The Mechanism of *recA polA* Lethality: Suppression by RecA-Independent Recombination Repair Activated by the *lexA*(Def) Mutation in *Escherichia coli*

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Manuscript received October 28, 1994
Accepted for publication January 7, 1995

ABSTRACT

The mechanism of *recA polA* lethality in *Escherichia coli* has been studied. Complementation tests have indicated that both the 5′ → 3′ exonuclease and the polymerization activities of DNA polymerase I are essential for viability in the absence of RecA protein, whereas the viability and DNA replication of DNA polymerase I-defective cells depend on the recombinase activity of RecA. An alkaline sucrose gradient sedimentation analysis has indicated that RecA has only a minor role in Okazaki fragment processing. Double-strand break repair is proposed for the major role of RecA in the absence of DNA polymerase I. The *lexA*(Def)::Tn*5* mutation has previously been shown to suppress the temperature-sensitive growth of *recA200*(Ts) *polA25*::*spc* mutants. The *lexA*(Def) mutation can alleviate impaired DNA synthesis in the *recA200*(Ts) *polA25*::*spc* mutant cells at the restrictive temperature. *recF*$^+$ is essential for this suppression pathway. *recJ* and *recQ* mutations have minor but significant adverse effects on the suppression. The *recA200*(Ts) allele in the *recA200*(Ts) *polA25*::*spc lexA*(Def) mutant can be replaced by Δ*recA*, indicating that the *lexA*(Def)-induced suppression is RecA independent. *lexA*(Def) reduces the sensitivity of Δ*recA polA25*::*spc* cells to UV damage by ~10^4-fold. *lexA*(Def) also restores P1 transduction proficiency to the Δ*recA polA25*::*spc* mutant to a level that is 7.3% of the *recA*$^+$ wild type. These results suggest that *lexA*(Def) activates a RecA-independent, RecF-dependent recombination repair pathway that suppresses the defect in DNA replication in *recA polA* double mutants.

THE RecA protein of *Escherichia coli* plays crucial roles in homologous recombination and recombination repair (WEST 1992; COX 1993; KOWALCZYKOWSKI *et al.* 1994). It has two enzymatic activities: a recombinase, which promotes homologous pairing and strand exchange between DNA molecules, and a coprotease, which facilitates the cleavage of LexA protein in SOS induction and lytic repressor in lambda prophage induction. The *recA* mutants defective in homologous recombination show increased sensitivity to UV light radiation (CLARK and MARGULIES 1965), lack of homologous recombination (CSONKA and CLARK 1979), and significant chromosome degradation (SKARSTAD and BOYE 1993). However, the *recA* gene normally is not essential for cell viability. Strains containing a *recA* deletion grow almost as well as the wild type does either in minimum or rich medium (WITKIN and ROEGNER-MANISCALCO 1992).

DNA polymerase I (DNA Pol I), encoded by the *polA* gene, is involved in joining of Okazaki fragments during discontinuous DNA replication and in gap-filling during DNA repair (KORNBERG and BAKER 1992). The molecule has three enzymatic activities: a DNA polymerization activity, a 5′ → 3′ exonuclease that removes nucleotides from the 5′ ends of DNA or RNA, and a 3′ → 5′ exonuclease that edits the 3′ terminal nucleotide of a nascent DNA strand (JOYCE and STEITZ 1987). A concerted action of the 5′ → 3′ exonuclease and the polymerization activities simultaneously removes DNA or RNA and replaces it with the DNA moiety (nick translation). Cells deprived of DNA Pol I activity are sensitive to UV radiation, and the joining of Okazaki fragments is remarkably slowed in *polA* mutant cells (OKAZAKI *et al.* 1971). The deletion of the entire *polA* gene is lethal to cells in rich medium but not in minimum medium. This conditional inviability can be cured by introduction of either the 5′ → 3′ exonuclease or the polymerization activity of DNA Pol I (JOYCE and GRINDLEY 1984).

It has been known for more than 20 years that *recA polA* double mutants are inviable (GROSS *et al.* 1971; MONK and KINROSS 1972), but the mechanism of this lethality has not been elucidated. *recA56* is a null mutation defective in both the recombinase and coprotease activities. *polA12* encodes a mutant DNA Pol I that is totally defective in nick translation at 42°. *recA56 polA12* mutants are temperature sensitive for growth. MONK and KINROSS (1972) showed that DNA synthesis in this double mutant gradually ceased at the nonpermissive temperature. They examined the rate of nascent DNA fragment joining by alkaline sucrose gradient sedimentation and concluded that the inviability of the double mutants is not due to a defect in DNA fragment joining

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(MONK *et al.* 1973). *recA718* mutants exhibit moderate UV sensitivity but are competent in homologous recombination. A *recA718 polA12* double mutant can grow slowly in minimum medium but cannot form colonies in rich medium at 42° (WITKIN and ROEGNER-MANISCALCO 1992). The joining of Okazaki fragments in this double mutant is slowed at the nonpermissive condition, but the introduction of mammalian DNA polymerase β enhances the joining rate (SWEASY and LOEB 1992). Moreover, WITKIN and ROEGNER-MANISCALCO (1992) showed that overproduction of DnaE protein can restore viability to *recA718 polA12* cells in rich medium at 42°. These recent results suggest that the most important function of DNA Pol I is the joining of Okazaki fragments during DNA replication. A proposed role of RecA is to perform postreplicational recombination repair of persistent gaps on the lagging strand (WITKIN and ROEGNER-MANISCALCO 1992).

The *polA25*::*spc* mutation has an insertion of a *mini*-Tn*10spc* at a site close to the 3′ end of the *polA* coding region (CAO *et al.* 1993). It encodes a mutant DNA Pol I that is thought to lack the polymerization activity but to retain both the exonuclease activities (CAO *et al.* 1993). A combination of this mutation and the *recA200*(Ts) mutation, which encodes a temperature-sensitive RecA, renders cells temperature sensitive for growth. We previously found that the *lexA*(Def) mutation suppresses the conditional lethality of the double mutants (CAO *et al.* 1993). The *lexA* defective mutation leads to derepression of the LexA regulon genes, many of which are involved in DNA replication, repair and recombination. This category of LexA regulon genes includes *recA, recQ, recN, ruvAB, polB, uvrA, dnaQ, dnaN* and *ssb* (WALKER 1984; WITKIN 1991). It seems that the derepression of one or more of the LexA regulon genes compensates for the defects of the *recA polA* double mutants in DNA replication.

In this study, we have examined the requirement for potential enzymatic activities of DNA Pol I in the complete absence of RecA protein, and *vice versa*. We have also examined the joining of nascent DNA fragments in a set of *recA polA* mutants. Based on the results, we suggest a mechanism for the lethality of the *recA polA* double mutants. Our characterization of the suppression in *recA200 polA25*::*spc* by *lexA*(Def) suggests the presence of an inducible RecA-independent homologous recombination pathway.

MATERIALS AND METHODS

Media and growth conditions: Unless otherwise stated, cells were grown at 37° in Luria broth (LB) or M9 salts-glucose minimal medium (M9G) (MILLER 1992) supplemented with Casamino Acids (CAA) (0.2%; Difco Laboratories, Detroit, MI), required amino acids (50 μg/ml) and thymine (8 μg/ml).

Chemicals and isotopes: The chemicals used were from Sigma Chemical (St. Louis, MO). Restriction enzymes were from GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD). [Methyl-^{3}H]thymine and [methyl-^{3}H]thymidine were from Du Pont Company (Boston, MA).

***E. coli* strains and plasmids:** The *E. coli* strains used in this study are listed in Table 1. Strains were constructed by phage P1*vir*-mediated transduction (LENNOX 1955).

The strains harboring F′ episomes that carry different parts of the *polA* gene and a Cm^r marker were from R. MAURER (LIFSICS *et al.* 1992). Plasmid pβL was constructed by J. SWEASY and obtained via R. MAURER (LIFSICS *et al.* 1992; SWEASY and LOEB 1992). pβL is a pHSG576 derivative (Cm^r) that contains gene encoding rat DNA polymerase β under the control of *lac* promoter. The strains harboring different miniF-*recA* alleles with a Km^r marker were from S. SOMMER (DUTREIX *et al.* 1989; ASAI *et al.* 1993).

Introduction of the plasmids: F′ episomes carrying different parts of *polA* were introduced to a Δ*polA recA200* strain (AQ9426) by mating in CAA medium at 30° for 1.5 hr. The exconjugants were then selected for Cm^r and Tc^r on CAA plates. The introduction of pβL was carried out by transforming AQ9426 to Cm^r at 30° on CAA plates. The construction of the Δ*recA polA12* miniF-*recA* strains was by transforming appropriate miniF-*recA* plasmid into a Δ*recA polA12* strain (AQ9507), selecting for Km^r.

Determination of surviving fractions: Viable counts were determined on LB plates at different temperatures as described previously (CAO *et al.* 1993). The surviving fraction was obtained by dividing viable counts by total cell numbers plated. Cell numbers were determined with a particle counter (ElectroZone, Particle Data, Elmhurst, IL).

Measurement of DNA synthesis: DNA synthesis was determined by measuring incorporation of [^{3}H]thymine into the acid insoluble fraction as described previously (KOGOMA and LARK 1975).

Alkaline sucrose gradient sedimentation analysis: The analysis was performed according to the procedure described previously (OKAZAKI *et al.* 1968; JACOBSON and LARK 1973; FIJALKOWSKA *et al.* 1989) with some modifications. Pulse-labeled cells were collected and resuspended in 40 μl ice-cold M9G containing thymidine (50 μg/ml). Samples were loaded onto 5–20% alkaline sucrose gradients (0.2 N NaOH, 0.8 M NaCl, 0.02 M EDTA) bottomed with 60% sucrose cushion (200 μl) and capped with 200 μl of lysis mix (1% SDS, 0.01 M EDTA, 0.25 M NaCl, 0.5 M NaOH). After 20 min at room temperature, samples were centrifuged in a SW50 rotor at 30,000 rpm for 2 hr at 20°. Fractions were collected from the bottom of the tubes onto 3MM paper filters, and the radioactivity in the acid-insoluble fraction was determined by scintillation counting.

UV sensitivity test: Sensitivity to UV light was determined by the method of LLOYD and BARBOUR (1974).

Measurement of P1 transduction frequency: The *asnA*::*cat* marker (84.3 min) was used in the transduction. The *asnA*::*cat* allele was constructed by inserting a *cat* cassette at the *Bam*HI site of *asnA*. The allele was transferred onto the chromosome by two *in vivo* double-crossover events, first into a λ phage vector and then to the chromosome (D. BATES, T. ASAI, and T. KOGOMA, unpublished data). The advantage of using the *asnA* marker was that there is another *asn* gene (*asnB*) in *E. coli* that can functionally substitute for *asnA*, leading to little bias in the transduction frequency. All recipient cells were *asnA*$^+$ and the transductants were selected for Cm^r, with $\sim 2 \times 10^9$ recipient cells spread on each plate. After 48 hr incubation at 30°, the transduction frequencies were determined.

Southern blot hybridization: Chromosomal DNA was extracted according to the method by WILSON (1987). DNA was digested with *Pst*I and electrophoresed in a 1% agarose gel. Then a blot was made and hybridized to a ^{32}P-labeled probe

TABLE 1

***E. coli* strains used in the study**

Strain	Relevant genotype	Source, reference or construction[a]
AQ377[b]	*recA200*	TORREY and KOGOMA (1982)
AQ663	*Δ(srlR-recA)306*	As JC10284 (CSONKA and CLARK 1979)
AQ3474[b]	*recA200 sfi11*	CAO *et al.* (1993)
AQ3476[b]	*recA200 lexA71*::Tn*5 sfi11*	CAO *et al.* (1993)
AQ4790	*recF322*::Tn*3*	This laboratory
AQ6039	*polA12*	MONK and KINROSS (1972)
AQ6375	Hfr, *thyA*$^+$	As CAG12200 (SINGER *et al.* 1989)
AQ8534	*polA25*::*spc zih-35*::Tn*10*	CAO *et al.* (1993)
AQ8619[b]	*recA200 polA25*::*spc lexA71*::Tn*5 sfi11*	P1.AQ8534 × AQ3476 → Tc^r, spc^r
AQ8633[b]	*recA200 polA25*::*spc sfi11*	P1.AQ8534 × AQ3474 → Tc^r, spc^r
AQ8695[b]	*recA200 polA*::*25spc lexA71*::Tn*5 sfi11*	As AQ8619 but Tc^s (CAO *et al.* 1993)
AQ8718[b]	*Δ(srlR-recA)306 lexA71*::Tn*5 sfi11*	P1.AQ663 × AQ3476 → Tc^r, UV^s
AQ8720	*lexA71*::Tn*5 malF3089*::Tn*10*	This laboratory
AQ8763[b]	*Δ(srlR-recA)306*	P1.AQ663 × AQ377 → Tc^r, UV^s at 30°
AQ8869[b]	*recA200 polA25*::*spc sfi11*	As AQ8633 but Tc^s (BOCHNER *et al.* 1980)
AQ8882[b]	*polA25*::*spc sfi11*	AQ6375 (Hfr) × AQ8869 → Thy$^+$, more UV^r at 42°
AQ8926[b]	*polA25*::*spc lexA71*::Tn*5 sfi11*	P1.AQ8720 × AQ8882 → Tc^r, Km^r
AQ8996[b]	*Δ(srlR-recA)306 polA25*::*spc lexA71*::Tn*5 sfi11*	P1.AQ663 × AQ8695 → Tc^r
AQ9016	*ΔpolA zih-35*::Tn*10*	CAO and KOGOMA (1993)
AQ9237[b]	*recA*$^+$ *sfi11*	This laboratory
AQ9334[b]	*recA200 polA25*::*spc lexA71*::Tn*5 recF322*::Tn*3 sfi11*	P1.AQ4790 × AQ8695 → Ap^r
AQ9352[b]	*recA200 recF322*::Tn*3 sfi11*	P1.AQ4790 × AQ3474 → Ap^r, more UV^s at 30°
AQ9354[b]	*polA25*::*spc recF322*::Tn*3 sfi11*	P1.AQ4790 × AQ8882 → Ap^r, more UV^s
AQ9426[b]	*ΔpolA recA200 sfi11*	P1.AQ9016 × AQ3474 → Tc^r, Ts, more UV^s
AQ9507	*Δ(srlR-recA)306 polA12*	P1.AQ663 × AQ6039 → Tc^r, Ts
AQ9511[b]	*recA200 polA*::*25spc lexA71*::Tn*5 sfi11* (λ-*rnhA*$^+$)	As AQ8619 but λ-*rnhA*$^+$
AQ9513[b]	*recA200 polA*::*25spc sfi11* (λ-*rnhA*$^+$)	As AQ8633 but λ-*rnhA*$^+$
AQ9826[b]	*recA200 polA*::*25spc lexA71*::Tn*5 recQ61*::*Tn3 sfi11*	P1.KD1996 × AQ8695 → Ap^r
AQ9854[b]	*recA200 polA*::*25spc lexA71*::Tn*5 recJ284*::Tn*10 sfi11*	P1.JC12123 × AQ8695 → Tc^r
AQ9857[b]	*recA200 polA*::*25spc lexA71*::Tn*5 ruvA60*::Tn*10 sfi11*	P1.N2057 × AQ8695 → Tc^r
JC12123	*recJ284*::Tn*10*	J. CLARK (LLOYD *et al.* 1987)
KD1996	*recQ61*::Tn*3*	H. NAKAYAMA (IRINO *et al.* 1986)
N2057	*ruvA60*::Tn*10*	R. LLOYD (SHURVINTON *et al.* 1984)

[a] Ts, temperature-sensitive growth. Tc^r, Ap^r, Km^r and spc^r represent resistance to tetracycline, ampiciline, kanamycin and spectinomycin, respectively. UV^s, sensitive to UV. UV^r, resistant to UV.

[b] The remaining genotype: *metE90 trpA9605 thy708 deo29 lacZ118 lacI22 rnhA102.*

as described by SAMBROOK *et al.* (1989). The probe was prepared by purifying the 1-kb *Mlu*I-*Mlu*I fragment of pOC162 (a kind gift from W. MESSER) and labeling with α-[^{32}P]dCTP (ICN Biomedicals, Inc., Irvine, CA) using the Random Primers Labeling Kit (BRL Life Technologies). The radioactive DNA bands were visualized by a PhosphorImager (425E-120, Molecular Dynamics, Sunnyvale, CA).

RESULTS

The nick translation activity of DNA Pol I is essential for viability in the absence of active RecA protein: *recA200* encodes a temperature-sensitive RecA, which has near normal RecA activity at 30° and confers the UV-sensitive and recombination-deficient phenotypes in the range of 35 to 42° (LLOYD *et al.* 1974). Therefore, the *recA200 ΔpolA* double mutant (AQ9426) is temperature sensitive for growth in minimal medium and is broth sensitive at all temperatures due to the *polA* deletion (CAO and KOGOMA 1993). Into this strain we introduced a set of F′ episomes that carry parts of the *polA* gene expressing different activities of DNA Pol I (Table 2). When the F′ plasmids confer either the 5′ → 3′ exonuclease or the polymerization activity, they could restore viability to the *recA200 ΔpolA* double mutant on rich medium at 30°. This confirms the previous result (JOYCE and GRINDLEY 1984). However, to restore viability to the double mutant at 42°, both the 5′ → 3′ exonuclease and polymerization activities were required (Table 2). The D355A E357A allele is a *polA* gene with two mutations that specifically affect the 3′ → 5′ exonuclease activity without inactivating the other two activities of DNA Pol I. This allele restored viability to the double mutant at 42°, indicating the absence of requirement for the 3′ → 5′ exonuclease activity. pβL, which is capable of replicating in *polA* mutants owing to the pSC101 replication origin, expresses mammalian DNA polymerase β (DNA Pol β). DNA Pol β has been shown to complement the defect of *polA12* in Okazaki fragment joining at the nonpermissive temperature de-

TABLE 2

Complementation of Δ*polA recA200* cells (AQ9426) by F′ episomes carrying different parts of the *polA* gene

Strain and plasmid	Activities in DNA Pol I[a] on F′ plasmid			Colony formation[b]			
				30°		42°	
	5′ → 3′ exonuclease	3′ → 5′ exonuclease	Polymerase	M9G	LB	M9G	LB
AQ9426	−	−	−	+	−	−	−
AQ9426 F′ 5′ → 3′ exo	+	−	−	+	+	−	−
AQ9426 F′ klenow	−	+	+	+	+	−	−
AQ9426 F′ D355A E357A	+	−	+	+	+	+	+

[a] +, activity present; −, activity not present.
[b] +, colonies formed; −, no colonies formed.

spite the fact that the enzyme lacks detectable exonuclease activities (Sweasy and Loeb 1992). Introduction of pβL plasmid into AQ9426 complemented the broth sensitivity at 30° but not the temperature-sensitive growth (data not shown). These results indicate that both the 5′ → 3′ exonuclease and polymerization activities, that is, the nick translation activity of DNA Pol I, is essential for viability in the complete absence of RecA. The polymerization or the 5′ → 3′ exonuclease activity of DNA Pol I alone is not sufficient to overcome the absence of functional RecA protein.

The survival of *polA* mutant cells depends on the recombinase activity of RecA: The *recA428* mutation confers severe deficiency in homologous recombination and a constitutive coprotease activity (Asai *et al.* 1993). The *recA730* mutation imparts recombination proficiency and a constitutive coprotease activity (Witkin *et al.* 1982). The *recA430* mutation causes partial defects in homologous recombination, SOS induction and prophage induction (Roberts and Roberts *et al.* 1981; Menetski and Kowalczykowski 1990). MiniF episomes carrying the above *recA* mutations and the wild-type *recA* gene were transformed into a Δ*recA polA12* strain (AQ9507). The viability of these transformants was then examined at 30 and 42° (Table 3). The miniF-*recA428* could not complement the temperature-sensitive growth of Δ*recA polA12*, whereas miniF-*recA*$^+$ or miniF-*recA730* improved the viability ≥35-fold at 30° and 10^4-fold at 42°. These results indicate that the inability of miniF-*recA428* to complement the *recA* defect is not due to the constitutively expressed coprotease activity of RecA but rather to the defective recombinase activity. Thus, the recombinase activity of RecA is essential for the survival of *polA* mutants. Furthermore, only a portion of the wild-type level of RecA recombinase activity is sufficient to restore the growth of *recA polA* double mutants because *recA430* could also alleviate the temperature sensitivity of Δ*recA polA12* despite the low level of recombinase activity.

TABLE 3

Complementation of Δ*recA polA12* cells (AQ9507) by different miniF-*recA* plasmids

Strain	*recA* allele on miniF plasmid	Plating efficiency on LB plates	
		30°	42°
AQ9507	None	9.9×10^{-3}	9.0×10^{-6}
AQ9538	*recA*$^+$	8.3×10^{-1}	9.4×10^{-1}
AQ9542	*recA428*	3.2×10^{-3}	4.5×10^{-6}
AQ9515	*recA730*	3.5×10^{-1}	1.5×10^{-1}
AQ9597	*recA430*	4.5×10^{-1}	7.7×10^{-2}

The RecA recombinase is essential for DNA synthesis in the absence of DNA Pol I: Next, we examined DNA synthesis at 30 and 42° in these strains (Figure 1). The Δ*recA polA12* derivative harboring miniF-*recA428* (AQ9542) synthesized DNA as efficiently as the miniF-*recA*$^+$ strain (AQ9538) did at 30°. However, at 42° DNA synthesis in AQ9542 as well as a Δ*recA* control (AQ9507) ceased after ~2 hr (Figure 1, inset). In contrast, DNA synthesis in the Δ*recA polA12* double mutant with miniF-*recA730* (AQ9515) or with the miniF-*recA430* (AQ9597) continued both at 30 and 42°. Thus, the recombinase activity of RecA is essential for the continuation of DNA synthesis in *polA* mutant cells. The level of DNA synthesis in the strains with *recA730* and with *recA430* even exceeded that with *recA*$^+$. The reason for this enhanced DNA synthesis is not clear at present.

To examine the rate of Okazaki fragment joining in the above strains at the nonpermissive temperature, cultures were shifted from 30 to 42° and were pulse-labeled with [^{3}H]thymidine after 10 min incubation at 42°. One half of the pulse-labeled samples was then chased with nonradioactive thymidine. The alkaline sucrose gradient sedimentation analysis (Figure 2) shows that the size of DNA fragments in the strain with *recA428* after pulse as well as after chase was slightly smaller than that seen with *recA*$^+$. However, there were no nascent DNA fragments accumulated in the *recA428* cells as indicated by comparison of fraction numbers 19–20 between the *recA428* and *recA*$^+$ samples, which correspond to 1–2 kb size of nascent DNA fragments. Therefore, accumulation of small DNA fragments is not the cause for the lethality of the *recA polA* double mutants.

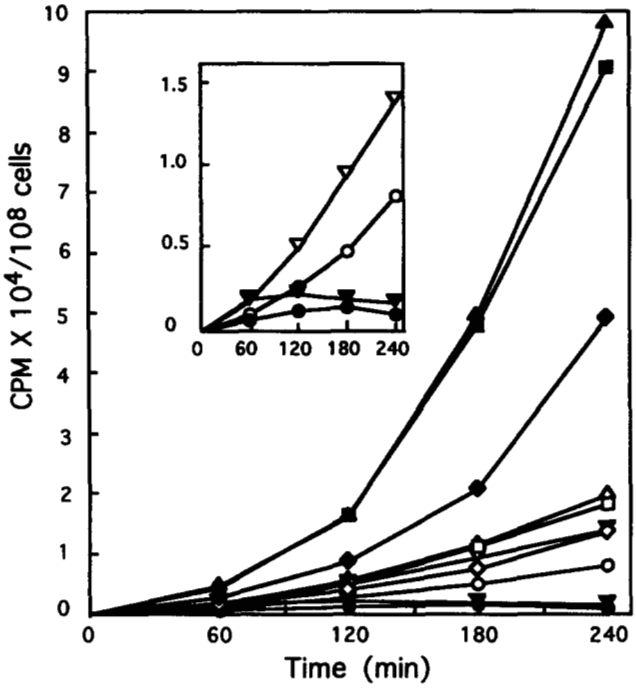


FIGURE 1.—Measurement of DNA synthesis in Δ*recA polA12* miniF-*recA* strains. Cells were grown in CAA medium at 30° until cell density reached 1–1.5 × 10^8 cells/ml. Then, [^{3}H]-thymine was added (to a final concentration of 10 μCi/8 μg/ml), and the culture was split into two halves: one half was incubated at 30° and the other at 42°. Samples were taken immediately (time zero) and every hour thereafter, and the incorporation of [^{3}H]thymine into the acid-insoluble fraction was determined. Open symbols, at 30°; closed symbols, at 42°. AQ9507 (Δ*recA*, ○ ●); AQ9542 (miniF-*recA428*, ▽ ▼); AQ9515 (miniF-*recA730*, △ ▲); AQ9597 (miniF-*recA430*, □ ■); AQ9538 (miniF-*recA*$^+$, ◇ ◆). The inset shows the details of change in DNA synthesis rates in AQ9507 and AQ9542 at 30 and 42°.

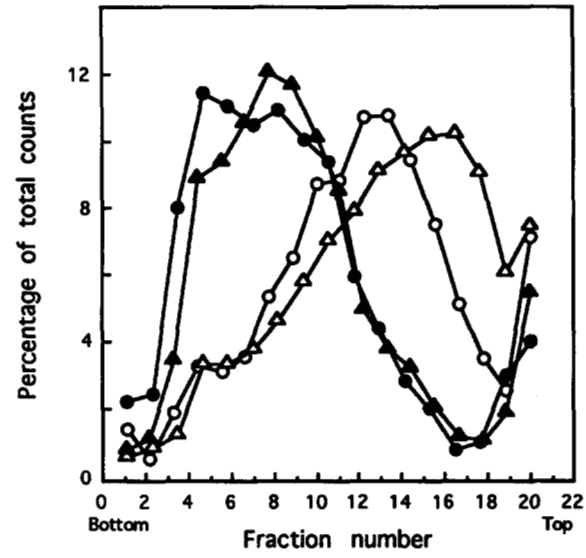


FIGURE 2.—Size of pulse-labeled DNA fragments in Δ*recA polA12* miniF-*recA428* cells. The experiments were performed as described in MATERIALS AND METHODS. A 1:25 dilution of overnight culture was inoculated into CAA medium. Cells were grown at 30° for 1.5–2 hr until cell density reached 1 × 10^8 cells/ml and then shifted to 42°. After incubation at 42° for 10 min, cells were pulse-labeled by placing 2 ml of the culture into prewarmed 200 μl of [^{3}H]thymidine (0.1 mCi/ml) at 42°. After 5 min, an aliquot (1 ml) of the pulse-labeled sample (nonchase) was transferred to 4 ml of ice-cold M9G with 0.01 M KCN to stop further incorporation. Another aliquot (1 ml) of the labeled culture was added to 4 ml of prewarmed M9G medium containing an excess of thymidine (50 μg/ml) and incubated at 42° for 15 min. The culture was then placed on ice (chase). Alkaline sucrose gradient sedimentation was performed as described in MATERIALS AND METHODS. Percentage of total counts was calculated by dividing the CPM of each fraction by the total CPM. Open symbols, nonchase; closed symbols, chase. AQ9538 (miniF-*recA*$^+$, ○ ●); AQ9542 (miniF-*recA428*, △ ▲). The total radioactive counts for the AQ9538 nonchase, AQ9538 chase, AQ9542 nonchase, and AQ9542 chase samples were 1.36 × 10^5, 1.14 × 10^5, 3.33 × 10^4, and 2.33 × 10^4 CPM, respectively.

The amount of DNA synthesized in the *recA428* cells was 68% of that with *recA*$^+$ for 5 min. The extent of nascent DNA stability was examined in the miniF-*recA* strains by measuring the amounts of CPM (radioactive counts per minute) before and after the 15-min chase. In the *recA*$^+$ cells, 84% of the 5-min pulse label was retained after the 15-min chase at 42°. Nascent DNA stability in the other strains at 42° was 70% with *recA428*, 68% with *recA730*, and 64% with *recA430*. Because the *recA730* and *recA430* cells were viable whereas the *recA428* cells were not, we conclude that DNA degradation is not the cause for the lethality of the *recA polA* double mutants.

***lexA*(Def)::Tn*5* can suppress the temperature-sensitive growth of *recA200 polA25*::*spc*:** The *polA25*::*spc* mutation is thought to inactivate the polymerization activity of DNA Pol I without affecting the exonuclease activities (CAO *et al.* 1993). The *lexA*(Def)::Tn*5* mutation restores viability to a *recA200 polA25*::*spc* double mutant at 42° (CAO *et al.* 1993). The viability of this suppressed strain (AQ8619) was ~10^4-fold higher than the unsuppressed counterpart (AQ8633) at 42° (Table 4). Since RecA200 protein is overproduced in *lexA*(Def) mutants, one possible mechanism for the suppression could be that overexpression of RecA200 protein, which might have a residual activity at 42°, overcame the defect. This possibility was ruled out by demonstration that the *recA200* allele could be replaced by the Δ*recA* allele (AQ8996 in Table 4). This suggests that a LexA regulon gene(s) other than *recA* is involved in the suppression.

To determine how well *lexA*(Def) can suppress the double mutant on the level of DNA synthesis, the DNA synthesis rate was measured by pulse labeling (Figure 3). The initial analysis with cells growing in liquid medium revealed that DNA synthesis measurement was compromised by a significant level of DNA degradation in these strains at the restrictive temperature. Therefore, we monitored DNA synthesis rates in cells growing on plates as described previously (WITKIN and ROEGNER-MANISCALCO 1992; CAO *et al.* 1993). The result indi-

TABLE 4

The plating efficiency of *recA* and *polA* strains

Strain	Relevant genotype				Plating efficiency on LB plates[a]	
	recA	*polA*	*lexA*	Others	30°	42°
AQ3474	*200*	+	+		$(4.0 \pm 0.1) \times 10^{-1}$	$(7.2 \pm 0.2) \times 10^{-1}$
AQ8882	+	*25*::*spc*	+		$(3.8 \pm 0.1) \times 10^{-1}$	$(3.6 \pm 0.8) \times 10^{-1}$
AQ8633	*200*	*25*::*spc*	+		$(3.3 \pm 0.7) \times 10^{-1}$	$(3.3 \pm 2.6) \times 10^{-5}$
AQ8619	*200*	*25*::*spc*	*71*::Tn*5*		$(1.1 \pm 0.8) \times 10^{-1}$	$(9.6 \pm 3.7) \times 10^{-2}$
AQ8996	Δ*306*	*25*::*spc*	*71*::Tn*5*		$(1.1 \pm 0.1) \times 10^{-1}$	$(8.9 \pm 1.3) \times 10^{-2}$
AQ9352	*200*	+	+	*recF*::Tn*3*	$(6.4 \pm 0.8) \times 10^{-1}$	$(4.2 \pm 0.7) \times 10^{-1}$
AQ9354	+	*25*::*spc*	+	*recF*::Tn*3*	$(3.7 \pm 0.5) \times 10^{-1}$	$(3.7 \pm 0.1) \times 10^{-1}$
AQ9334	*200*	*25*::*spc*	*71*::Tn*5*	*recF*::Tn*3*	$(4.5 \pm 2.0) \times 10^{-1}$	$(5.6 \pm 0.2) \times 10^{-5}$
AQ9854	*200*	*25*::*spc*	*71*::Tn*5*	*recJ*::Tn*10*	$(1.7 \pm 0.1) \times 10^{-1}$	$(1.6 \pm 0.1) \times 10^{-2}$
AQ9857	*200*	*25*::*spc*	*71*::Tn*5*	*ruvA*::Tn*10*	$(2.4 \pm 0.5) \times 10^{-1}$	$(1.4 \pm 0.6) \times 10^{-1}$
AQ9826	*200*	*25*::*spc*	*71*::Tn*5*	*recQ*::Tn*3*	$(2.8 \pm 0.7) \times 10^{-1}$	$(3.0 \pm 0.7) \times 10^{-1}$

[a] An average of two determinatons ± SEM.

cates that DNA synthesis rates were similar in the suppressed [AQ9511, *recA200 polA25*::*spc lexA*(Def)] and unsuppressed strain (AQ9513, *recA200 polA25*::*spc lexA*$^+$) during the first hour. DNA synthesis in AQ9513 gradually ceased after ~4 hr while in AQ9511 it continued. This result suggests that *lexA*(Def) restores DNA synthesis in *recA polA* double mutant cells in the restrictive condition.

The size of DNA fragments synthesized in the unsuppressed and suppressed strains was also examined (Figure 4). By calculating the total incorporation of [^{3}H]-thymidine in a 10-min pulse, we determined that the DNA synthesis rate in the suppressed strain improved by about fivefold over the unsuppressed and recovered to a level that was 30% of the *recA*$^+$ control level (Figure 4A). The joining of DNA fragments in the suppressed strain was only slightly improved over the unsuppressed (Figure 4B). These results suggest that derepression of one or more LexA regulon gene(s) results in activation of a pathway that suppresses the DNA replication defect in *recA polA* double mutants. We designated it the Srp pathway for suppression of the *recA polA* lethality.

***recF*$^+$ is essential for the Srp pathway:** The requirement for the RecA recombinase activity in *polA* mutants suggests that the Srp pathway might involve homologous recombination activity independent of RecA. Because some of the genes involved in the *recF* recombination pathway, such as *recN*, *recQ* and *ruvA*, are under LexA control, we examined a possible involvement of *recF*$^+$ in the Srp pathway. The *recF322*::*Tn3* mutation was introduced into the temperature-resistant strain *recA200 polA25*::*spc lexA*(Def) (AQ8695). The resulting strain, AQ9334, was temperature sensitive for growth (Table 4). The growth failure of this strain at the restrictive temperature is due neither to the combination of the *recF* and *polA* mutations (AQ9354) nor to the combination of *recF* and *recA*(Ts) (AQ9352) as indicated by the fairly good viability of these double mutants (Table 4). Therefore, *recF*$^+$ is essential for the Srp pathway.

Effects of *recJ*, *ruvA* and *recQ* mutations on the Srp pathway: We also introduced *recJ284*::*Tn10*, *ruvA60*::*Tn10* and *recQ61*::*Tn3*, into the *recA200 polA25*::*spc lexA*(Def) strain to see if RecJ, RuvA and RecQ, which are normally involved in the RecF recombination pathway, play any roles in the Srp pathway. The viability of a *ruvA60*::*Tn10* derivative (AQ9857) at 42° was almost as good as that at 30°, whereas the viability of *recJ284*::*Tn10* derivative (AQ9854) decreased 10-fold at 42°. The *recQ61*::*Tn3* derivative (AQ9826) formed about the same number of colonies at 42° as it did at

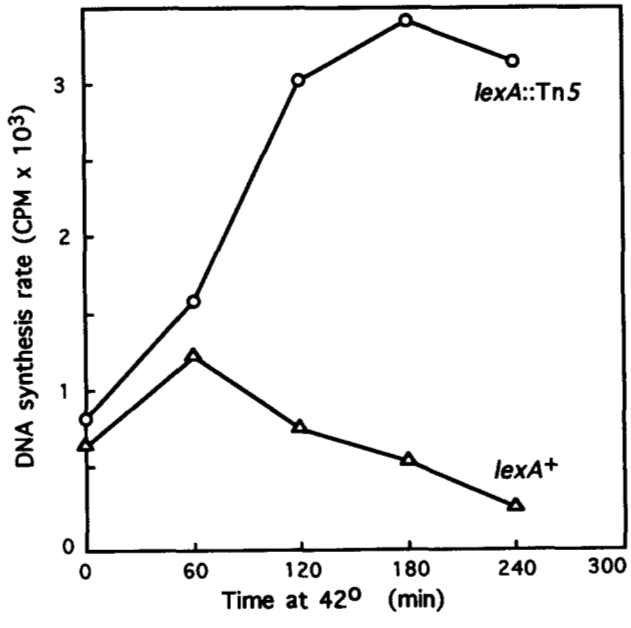


FIGURE 3.—The effect of *lexA*(Def) on DNA synthesis in *recA200 polA25*::*spc* cells. Cells were grown in LB medium at 30° for 2 hr until cell density reached 1 × 10^8 cells/ml, and the rate of DNA synthesis at 42° was determined on nutrient agar plates as described previously (CAO and KOGOMA 1993). The cells were pulse-labeled with [^{3}H]thymidine (10 μCi/0.12 μg/ml) for 10 min. △, AQ9513 (*lexA*$^+$); ○, AQ9511 [*lexA*-(Def)].

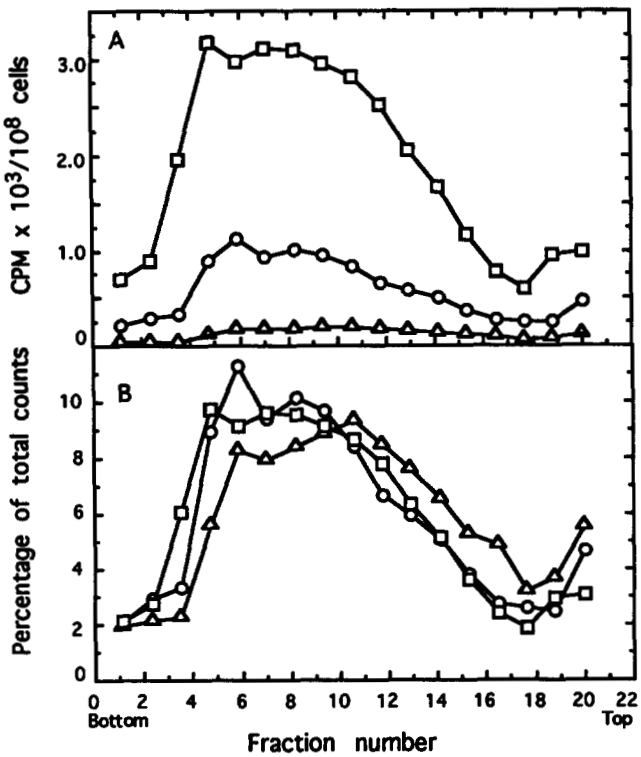


FIGURE 4.—Alkaline sucrose gradient sedimentation analysis of nascent DNA fragments in the *recA200 polA25*::*spc lexA*(Def) mutant. Cells were refreshed in LB medium at 30° for 2 hr until the cell density reached 1×10^8 cells/ml. An aliquot (100 μl) of refreshed cultures were plated on duplicated nutrient agar plates. After 1 hr incubation at 30°, plates were shifted to 42° and incubated for another hour. Then cells were pulse-labeled by spreading 30 μl of prewarmed [^{3}H]thymidine (0.1 mCi/ml) on duplicate plates. The plates were returned to 42°. After 10 min, the labeling was stopped by collecting the cells on the plate surface with 300 μl of cold M9G medium supplemented with thymine (50 μg/ml). The alkaline sucrose gradient sedimentation analysis was performed as described in MATERIALS AND METHODS. (A) a plot of CPM per 10^8 cells *vs.* the fraction number; (B) a plot of percentage of total CPM *vs.* the fraction number using the data in A. The total radioactive counts of the AQ9495 (*polA25*::*spc*, □), AQ9513 (*recA200 polA25*::*spc*, △), and AQ9511 [*recA200 polA25*::*spc lexA*(Def), ○] samples were 3.24×10^4, 2.09×10^3, and 9.93×10^3 CPM, respectively. The *rnhA102* mutation in these strains was complemented by lysogenization of a lambda phage carrying an *rnhA*$^+$ gene (Table 1).

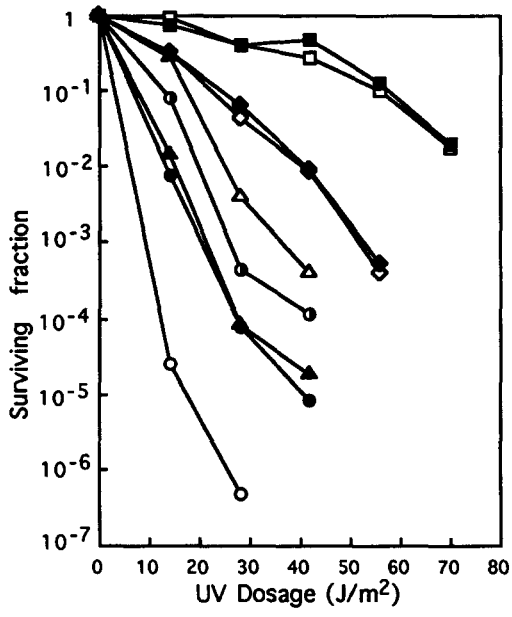
30°. However, the colonies at 42° were smaller and of varying sizes and shapes. These results suggest that *recJ*$^+$ and *recQ*$^+$ have some roles in the Srp pathway, whereas *ruvA*$^+$ is dispensable.

***lexA*(Def) increases resistance to UV damage in Δ*recA* cells:** The effect of *lexA*(Def) on the sensitivity to UV light was examined in *recA*$^+$, *recA200*, Δ*recA* cells at 30° (Figure 5). At a UV dosage of 25 J/m^2, *lexA*(Def) decreased sensitivity to UV light by 1000-fold in Δ*recA* cells (AQ8718 *vs.* AQ8763). The presence of *polA25*::*spc* further decreased sensitivity to UV light by another 10-fold (AQ8996 *vs.* AQ8718). These results indicate that derepression of the LexA regulon results in an increase in DNA damage repair capacities in Δ*recA* cells and that it is enhanced by the deficiency in DNA Pol I activity. These results support the notion that *lexA*(Def) activates a RecA-independent recombination repair pathway.

Interestingly, *lexA*(Def) showed no effect on sensitivity to UV damage in *recA200 polA*$^+$ at 30° (AQ3476 *vs.* AQ3474) or *recA*$^+$ *polA25*::*spc* cells (AQ8926 *vs.* AQ8882). On the other hand, the UV resistance of the *recA200 polA25*::*spc lexA*(Def) strain (AQ8619) was ~10-fold higher at 42° than at 30° (data not shown). These observations suggest that the RecA-independent recombination repair pathway does not function in the presence of a functional RecA protein. Furthermore, in *recA200 polA25*::*spc* cells, *lexA*(Def) increased the sensitivity to UV damage at 30° (AQ8619 *vs.* AQ8633). A possible reason for the negative effect of *lexA*(Def) on the repair of UV damage in *recA200 polA25*::*spc* is that the Srp pathway interferes with the RecA-dependent recombination repair pathway. The results in Figure 5 also show that in the absence of an active DNA Pol I, *recA200* mutant cells (AQ8633) are more sensitive to UV damage than *recA*$^+$ cells (AQ8882), suggesting that RecA200 protein is not as active as RecA$^+$ even at the permissive temperature, 30°.

A RecA-independent homologous recombination pathway can be induced by derepression of the LexA regulon: Homologous recombination proficiency was examined to see whether the suppression of Δ*recA polA25*::*spc* by *lexA*(Def) is accompanied by an increase in homologous recombination activity. Because the suppression by *lexA*(Def) can occur in the absence of RecA protein, we determined P1 transduction frequency in Δ*recA* strains combined with *lexA*(Def) or with *lexA*$^+$ using the *asnA*::*cat* marker (Table 5). A *recA* deletion mutation reduces the P1 transduction frequency to 100- to 1000-fold (HERTMAN and LURIA 1967). In our system, Δ*recA306* (AQ8763) reduced the frequency by ~1000-fold compared with *recA*$^+$ (AQ9237) (Table 5). The introduction of *lexA*(Def) into the Δ*recA* cells (AQ8718) slightly increased the P1 transduction frequency. However, this recombination activity was still 1000-fold lower than that of *recA*$^+$ cells. Only when Δ*recA* was combined with a defective *polA* gene (AQ8996) was this induced recombination activity significantly elevated. The induced RecA-independent recombination activity had 7.3% of the RecA-dependent activity (Table 5). This result indicates that a RecA-independent recombination pathway is induced by derepression of the LexA regulon in the Δ*recA polA25*::*spc* cells. This is a reminiscent of the effect of *lexA*(Def) on UV damage described above (Figure 5).

To determine whether these Cm^r transductants resulted from homologous recombination in the absence of RecA, we carried out Southern blot hybridization to examine the structure of the *asnA* locus in the transductants (Figure 6). With a DNA fragment containing part



	Strain	Relevant genotype		
		recA	*polA*	*lexA*
□	AQ3474	*200*	+	+
■	AQ3476	*200*	+	Tn*5*
◇	AQ8882	+	*spc*	+
●	AQ8926	+	*spc*	Tn*5*
△	AQ8633	*200*	*spc*	+
▲	AQ8619	*200*	*spc*	Tn*5*
○	AQ8763	Δ	+	+
●	AQ8718	Δ	+	Tn*5*
◐	AQ8996	Δ	*spc*	Tn*5*

FIGURE 5.—The effect of *lexA*(Def) on sensitivity to UV light. Viable cell counts were determined by plating at 30° after exposure to varying UV doses as described previously (LLOYD and BARBOUR 1974). The surviving fraction is the ratio of the viable counts of irradiated cells to that of unirradiated cells. The relevant genotypes of the tested strains are listed on the right of the plot. The average of the standard error of means was 13.5%.

of the *asnA* gene as a probe, the *asnA*$^+$ allele in Δ*recA* *lexA*(Def) and Δ*recA* *polA25*::*spc* *lexA*(Def) (Figure 6B, lanes 4 and 6) was found to be replaced by the *asnA*::*cat* allele in the Cm^r transductants (lanes 5, 7, and 8). The only Cm^r transductant obtained from Δ*recA* *lexA*$^+$ strain (AQ8763) (see Table 5) exhibited both the *asnA*$^+$ and *asnA*::*cat* alleles (lane 3), indicating that there was a nonhomologous recombination event, perhaps insertion, that occurred during transduction of AQ8763. These results suggest that the RecA-independent recombination activated by *lexA*(Def) appears to be a homologous recombination pathway.

DISCUSSION

The roles of DNA Pol I and RecA and the mechanism of the lethality: In the semidiscontinuous mode of DNA synthesis, one strand (the leading strand) is synthesized continuously while the other (the lagging strand) is synthesized in small pieces, Okazaki fragments (KORNBERG and BAKER 1992). The synthesis of each Okazaki fragment is primed by a small RNA (KITANI *et al.* 1985; ZECHNER *et al.* 1992). Because ligase cannot join RNA to DNA (WESTERGAARD *et al.* 1973), the RNA must be removed and replaced with the DNA moiety before ligase joins these fragments. Our results clearly indicate that in the absence of RecA function, both the 5′ → 3′ exonuclease and polymerization activities of DNA Pol I are essential for viability (Table 2). This is consistent with the assigned role of DNA Pol I in the Okazaki fragment processing, that is, the nick-translation that simultaneously removes RNA primers and fills with DNA (KORNBERG and BAKER 1992).

In the absence of DNA Pol I nick-translation activity (*e.g.*, in *polA12* cells at 42°), Okazaki fragment processing is not completely blocked. The nascent DNA is continuously converted to alkaline-resistant high molecular weight molecules (Figure 2) (MONK *et al.* 1973). This implies that other factors such as the RNase H activity of exonuclease III (KORNBERG and BAKER 1992), RNase HI (the *rnhA* gene product) and the α subunit (DnaE protein) of DNA Pol III can process Okazaki

TABLE 5

The P1 transduction frequency in Δ*recA* strains

Recipient strain	Relevant genotype			Plating efficiency[a] on LB plates at 30°	No. of Cm^r transductants in 1.2×10^{10} recipient cells[b]	Transduction frequency[c]
	recA	*polA*	*lexA*			
AQ9237	+	+	+	0.55 ± 0.04	3312 (1544, 1768)	5.0×10^{-7}
AQ8763	Δ	+	+	0.17 ± 0.02	1[d] (0, 1)	5.0×10^{-10}
AQ8718	Δ	+	Tn*5*	0.31 ± 0.04	2 (0, 2)	5.4×10^{-10}
AQ8996	Δ	*spc*	Tn*5*	0.12 ± 0.03	53 (26, 27)	3.7×10^{-8}

[a] An average of two determinations ± SEM.
[b] The sum of two independent experiments.
[c] The transduction frequency was calculated by dividing the number of transductants by the number of viable recipient cells plated.
[d] This transductant is most likely to have resulted from nonhomologous recombination; see Figure 6.

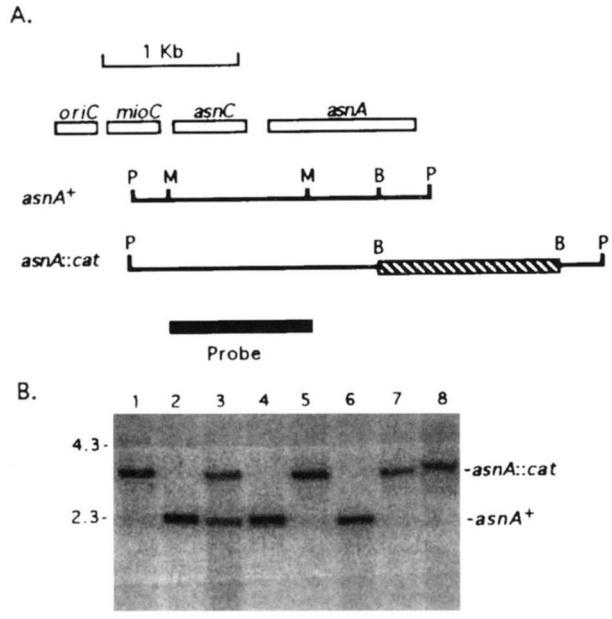


FIGURE 6.—Analysis of the Cm^r transductants by Southern blot hybridization. (A) The structure of the *asnA*$^+$ and *asnA*::*cat* genes. The restriction maps of the *asnA*$^+$ and *asnA*::*cat* alleles are shown relative to the *asnA* locus (BUHK and MESSER 1983). The striped bar is the *Bam*HI-*Bam*HI *cat* cassette from Tn*9* (KLECKNER *et al.* 1991), and the solid bar is the *Mlu*I-*Mlu*I fragment used as the probe for Southern blot hybridization. P, *Pst*I; M, *Mlu*I; B, *Bam*HI. (B) An autoradiogram of the blot. Lane 1, a Cm^r transductant of AQ9237 (*recA*$^+$ control). Lane 2, AQ8763 (Δ*recA*). Lane 3, a Cm^r transductant of AQ8763. Lane 4, AQ8718 [Δ*recA lexA*(Def)]. Lane 5, a Cm^r transductant of AQ8718. Lane 6, AQ8996 [Δ*recA polA25*::*spc lexA*(Def)]. Lane 7 and 8, two Cm^r transductants of AQ8996. Molecular weight markers are shown in kilobases.

fragments, albeit inefficiently. The involvement of DnaE protein has been supported by the observations that an excess of DnaE protein enhances gap filling *in vitro* (MAKI *et al.* 1985) and that overproduction of DnaE protein restores viability to *recA718 polA12* mutant cells in rich medium at 42° (WITKIN and ROEGNER-MANISCALCO 1992). Also, deletion of the entire *polA* gene is not lethal to the cells when cells are growing in minimal medium, suggesting the presence of a secondary, less efficient processing system (JOYCE and GRINDLEY 1984).

We have demonstrated that the recombinase, not the coprotease activity, of RecA is essential for viability of *polA12* mutants at 42°. This observation clearly rules out the possibility that the essential function of RecA in the absence of DNA Pol I activity is induction of the SOS response. The Δ*recA polA12* mutant harboring miniF-*recA428* exhibited an extent of nascent DNA loss that is similar to the loss seen in the counterparts harboring miniF-*recA730* or miniF-*recA430*. Thus, it is not likely that RecA is necessary for stabilization of nascent DNA before processing. RecA may be essential for filling the accumulating gaps on the lagging strand by recombinational repair using the newly synthesized daughter strand as template (WITKIN and ROEGNER-MANISCALCO 1992). However, this does not seem to be a major RecA role because fragment joining is only slightly slowed in the *recA428* mutant compared with *recA*$^+$ control cells (Figure 2).

We wish to propose double strand break (DSB) repair for a major role of RecA protein. In the absence of the nick-translation activity of DNA Pol I, the newly synthesized lagging strand is expected to exhibit a few nicks and gaps (Figure 7). When the next round of replication reaches the region and the leading strand replisome encounters a nick or a gap, a DSB would result. As previously discussed (ASAI *et al.* 1994; see also ROSENBERG and HASTINGS 1991), the broken chromosome arm would be degraded by the RecBCD enzyme to a nearest χ site to generate invasive single-stranded (ss) DNA. RecA protein would assimilate the ss DNA into a homologous duplex to yield a D-loop. The replisome assembly at the D-loop would successfully restore the replication fork, and the resolution of the Holiday junction wound finally resume the original chromosome structure (Figure 7). This proposal is consistent with the fact that the combination of *polA* and *recB* mutations is also lethal (MONK and KINROSS 1972). Furthermore, the model satisfactorily explains why DNA synthesis ceases only slowly upon loss of the DNA Pol I and RecA recombinase activities (Figure 1) (MONK and KINROSS 1972). The distance between a leading replication fork and the following fork depends on how often chromosome replication is initiated during a cell cycle (VON MEYENBURG and HANSEN 1987). In cells growing in minimal medium, a new round of replication is initiated when the leading fork travels halfway on the chromosome. Thus, there would be at least one-half generation time before the replication reaches the region with gaps and nicks. On the other hand, when cells are growing in rich medium, initiation occurs much more frequently, and subsequently the distance between the two forks is much shorter. This can explain why deletion of the entire *polA* gene is lethal in rich medium whereas it is not in minimal medium (JOYCE and GRINDLEY 1984). In minimal medium, the secondary processing system could have sufficient time to minimize the number of nicks and gaps, causing infrequent DSBs that could still be repaired by the RecA-dependent DSB repair (Figure 7). On the other hand, cells growing in rich medium have only a short time to process Okazaki fragments such that the number of DSBs overwhelms the DSB repair capacity, leading to cell death.

On the basis of these considerations we propose that the lethality of the *recA* and *polA* combination results from the failure to repair DSBs generated on the chromosomes that contain nicks and gaps due to inefficient Okazaki fragment processing in the absence of the nick-translation activity of DNA Pol I.

Suppression of the lethality by *lexA*(Def): We have discovered that the *lexA*(Def) mutation suppresses the *recA polA* lethality (Table 4). Thus, Δ*recA polA25*::*spc*

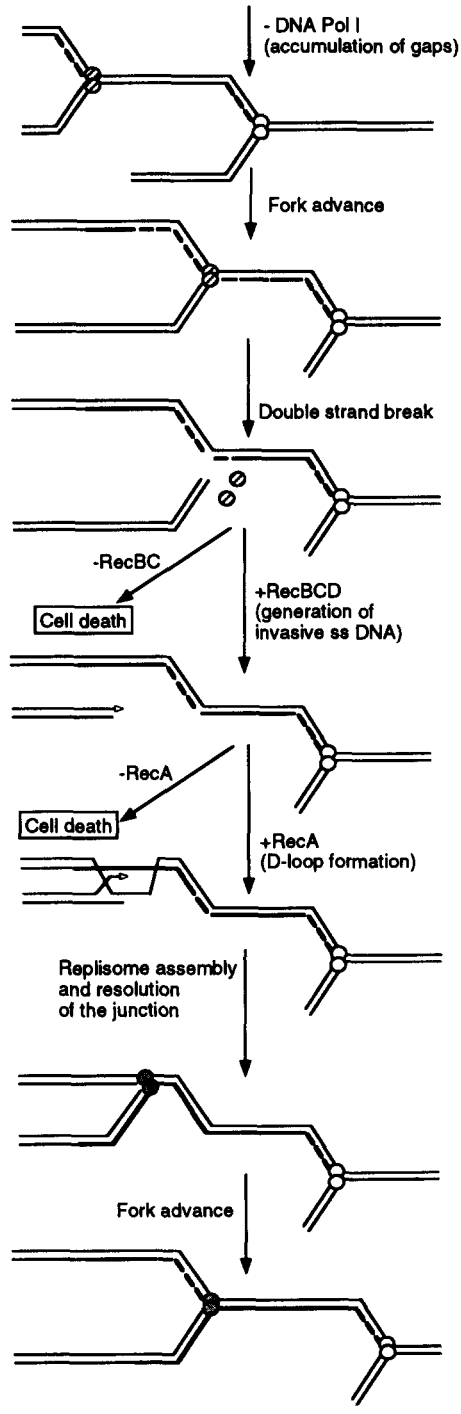


FIGURE 7.—The mechanism of the *recA polA* lethality. The region of nick or gap accumulation is shown in thick broken lines for emphasis. Slow repair of nicks and gaps by the secondary processing system (see the text) is assumed. The double spheres and the thin line with an open arrow represent a dimerized replisome and invasive ss DNA, respectively. For the details of D-loop formation and replisome assembly at the D-loop, see ASAI *et al.* (1994).

lexA(Def) triple mutants are viable and *recA200*(Ts) *polA25*::*spc* *lexA*(Def) is not temperature sensitive for growth. This implies that derepression of one or more LexA regulon genes (*srp*) alleviates the defects in the RecA recombinase activity or the DNA Pol I nick-translation activity or both. The derepression of the *srp* gene(s) by *lexA*(Def) allows DNA synthesis to continue in *recA200 polA25*::*spc* cells at 42° (Figure 3). It is unlikely that the suppression results from enhancement of Okazaki fragment processing because the joining of DNA fragments is only slightly slower in *lexA*$^+$ cells than in *lexA*(Def) cells (Figure 4).

One remarkable observation we have described in this report is that the *lexA*(Def) mutation restores to Δ*recA* mutants the ability to recombine with the chromosome a DNA fragment introduced by P1 phage (Table 5). Under certain specific conditions, the P1 transduction frequency in the complete absence of RecA can be elevated up to >7% of the *recA*$^+$ level. Under a similar condition, the *lexA*(Def) mutation renders Δ*recA* cells 1000-fold more resistant to UV radiation (Figure 5), suggesting an enhancement of recombination repair capacity. These observations point to the likelihood that derepression of the LexA regulon alleviates the defect in the RecA recombinase. We suggest that *lexA*(Def) activates a RecA-independent homologous recombination pathway. The *lexA*(Def) mutation cannot suppress the lethality of *recB polA* double mutants (data not shown). This indicates that the suppression is rather specific to the RecA defect and suggests that the derepression of the LexA regulon activates a RecA-like activity.

The nature of the RecA-independent homologous recombination pathway is not understood at present. Evidence suggests that the pathway does not function if functional RecA protein is present and that it interferes with the RecA-dependent recombination repair. The pathway is stimulated by the presence of *polA25*::*spc* as seen in both the P1 transduction frequency (Table 5) and the UV resistance (AQ8718 *vs.* AQ8996 in Figure 5). Since the mutant DNA Pol I (*polA25*::*spc*) is thought to be defective only in the polymerization activity (CAO and KOGOMA 1993), it suggests that the presence of gaps on the lagging strand stimulates the pathway. The proposed RecA-independent homologous recombination pathway greatly depends on *recF*$^+$. The *recJ*::Tn*10* and *recQ*::Tn*3* mutations have minor but significant adverse effects on the pathway. Recently, LOVETT *et al.* (1993) reported RecA-independent, homology-dependent deletion events and suggested that deletions result from misalignment between small homologous sequences during postreplication repair that occurs at or near a replication fork. Whether or not the RecA-independent deletion events depend on *recF*$^+$ is not known.

The candidates for an *srp* gene include *recQ, recN, ruvAB, polB, uvrA, dnaQ, dnaN* and *ssb*, which are under LexA control (WITKIN 1991). We have tested high copy-number plasmids carrying *recQ*$^+$, mimicking derepression by *lexA*(Def) and found no suppression of the *recA200 polA25*::*spc* lethality (data not shown). High

copy-number plasmid carrying *ssb*$^+$ could not transform the double mutants, perhaps because overproduction of Ssb protein is toxic to the cells. A low copy-number plasmid (a derivative of pSC101; 5–8 copies per cell) carrying *ssb*$^+$ did not suppress the lethality (data not shown). We are currently screening an *E. coli* genomic library constructed on a high copy-number plasmid for the Srp activity. Elucidation of the mechanism of *lexA*-(Def)-mediated suppression of the *recA polA* lethality awaits the identification and characterization of the *srp* gene product.

We are grateful to P. FOSTER, R. LLOYD, R. MAURER, W. MESSER, H. NAKAYAMA, S. SANDLER, S. SOMMER, and J. SWEASY for the gifts of *E. coli* mutant strains, plasmids and λ phages. This work was supported by Public Health Service grant GM-22092 from the National Institutes of Health and by grant BIR-9218818 from the National Science Foundation.

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Communicating editor: R. MAURER