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The DNA Replication Priming Protein, PriA, Is Required for Homologous Recombination and Double-Strand Break Repair

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The PriA protein, a component of the ϕX174-type primosome, was previously shown to be essential for damage-inducible DNA replication in *Escherichia coli*, termed inducible stable DNA replication. Here, we show that *priA*::*kan* null mutants are defective in transductional and conjugational homologous recombination and are hypersensitive to mitomycin C and gamma rays, which cause double-strand breaks. The introduction of a plasmid carrying the *priA300* allele, which encodes a mutant PriA protein capable of catalyzing the assembly of an active primosome but which is missing the n′-*pas*-dependent ATPase, helicase, and translocase activities associated with PriA, alleviates the defects of *priA*::*kan* mutants in homologous recombination, double-strand break repair, and inducible stable DNA replication. Furthermore, *spa-47*, which was isolated as a suppressor of the broth sensitivity of *priA*::*kan* mutants, suppresses the Rec$^-$ and mitomycin C sensitivity phenotypes of *priA*::*kan* mutants. The *spa-47* suppressor mutation maps within or very near *dnaC*. These results suggest that PriA-dependent primosome assembly is crucial for both homologous recombination and double-strand break repair and support the proposal that these processes in *E. coli* involve extensive DNA replication.

Homologous recombination, a ubiquitous activity in both prokaryotes and eukaryotes, not only is a means by which to generate genetic diversity but also plays a crucial role in the repair of DNA damage, including double-strand breaks (DSBs) (8, 12). Despite recent advances in our understanding of the mechanism of homologous recombination, the extent to which DNA synthesis might be involved in the process is largely unknown. PriA, a component of the priming system which primes DNA synthesis in the initiation of ϕX174 phage and ColE1-type plasmid DNA replication, has recently been shown to play an essential role in the initiation of DNA damage-inducible chromosome replication in *Escherichia coli*, termed inducible stable DNA replication (iSDR) (25). Here, we show that *priA* null mutants are defective in homologous recombination and are hypersensitive to chemical and physical agents that cause DSBs. These results strongly support the notion that extensive DNA replication is involved in homologous recombination and DSB repair.

The ϕX174-type primosome, originally discovered in the study of the initiation of phage ϕX174 DNA replication, consists of several *E. coli* proteins (see reference 23 for a review). PriA protein binds to a hairpin structure called n′-*pas* (primosome assembly site). PriB protein then binds to the PriA-DNA complex, and a single DnaB helicase is delivered from a DnaB-DnaC complex to the PriA-PriB-DNA complex by the action of DnaT (1). This step also involves PriC protein. DnaG primase then interacts with the DnaB and synthesizes the first RNA primer for the DNA polymerase III holoenzyme (replisome). In addition to the primosome assembly function, PriA exhibits *pas*-dependent ATPase, 3′→5′ helicase, and 3′→5′ translocase activities (reference 39 and references therein). However, the primosome assembly function of PriA can be uncoupled from the ATPase, helicase, and translocase activities. Thus, a mutant PriA protein (K230R), which is encoded by the *priA300* allele and has an amino acid substitution of arginine for lysine at the 230th residue, is completely deficient in these enzymatic activities, and yet it is capable of catalyzing the assembly of an active primosome in vitro (39). Since *priA*::*kan* mutants are viable, the ϕX174-type primosome is not absolutely essential for *E. coli* chromosome replication, although *priA*::*kan* mutants show reduced viability (18, 29), cell filamentation (29), hypersensitivity to UV radiation (18), and sensitivity to rich media (25). Moreover, *priA*::*kan* mutants cannot support the replication of ColE1-type plasmids (18, 25). The introduction of a plasmid carrying the *priA300* allele into a *priA2*::*kan* mutant has been shown to restore normal growth and nonfilamentous morphology (39).

Smith and coworkers proposed a model for homologous recombination in the RecBCD pathway (35–37). According to this model, the ends of a donor linear duplex DNA fragment introduced into cells are processed by the RecBCD enzyme to yield single-strand DNA with 3′ ends (for details, see reference 16). The 3′ ends are assimilated into the homologous regions of a recipient chromosome by the action of RecA, yielding a D-loop at each end. The D-loops develop into Holliday junctions, the subsequent resolution of which leads to reciprocal exchange resulting in integration of the donor DNA into the recipient chromosome and regeneration of a linear fragment which now contains the recipient sequence. Smith (36) pointed out that this reciprocity poses a potential problem because the linear DNA could continue to be engaged in the process indefinitely unless it is degraded or nonreciprocal exchange occurs. He offered a solution to this dilemma by proposing the possible conversion of the D-loop into a replication fork (36). We have proposed a specific mechanism for the initiation of DNA replication at a D-loop (2, 25). PriA-catalyzed primosome assembly is proposed to occur on the exposed single strand in the D-loop (Fig. 1). This step accomplishes DnaB

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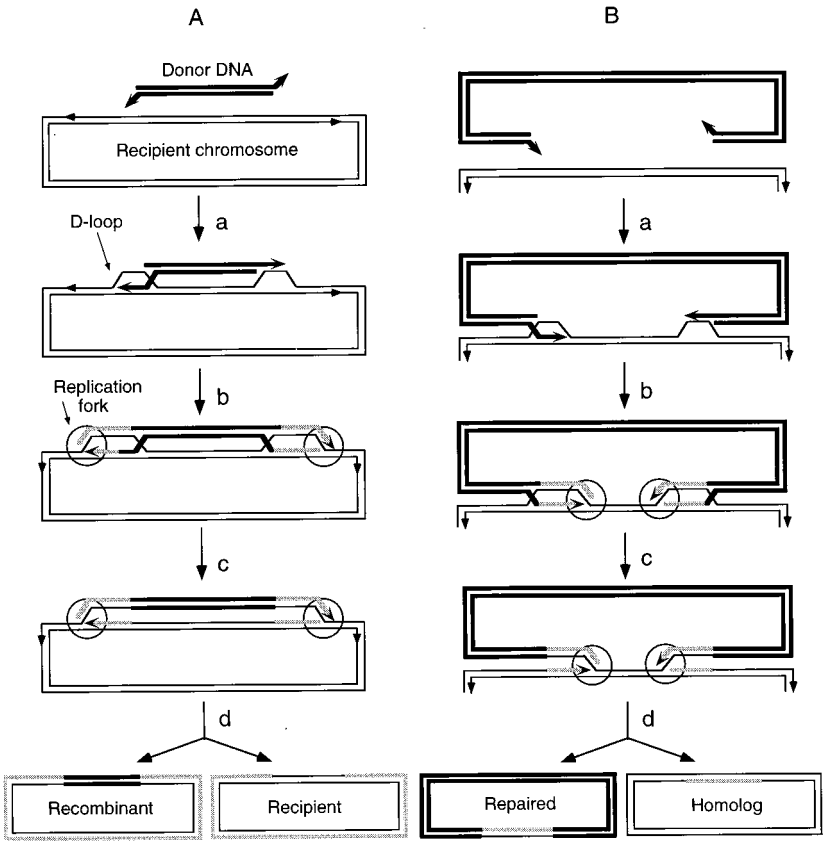

FIG. 1. Models for homologous recombination (A) and DSB repair (B). The ends of a linear DNA fragment (A; thick lines) or of a chromosome that has suffered a DSB (B; thick lines) are processed by the action of the RecBCD enzyme to yield single-stranded tails with 3′ OH ends (denoted by arrowheads). The tails are assimilated by the action of RecA into homologous regions of a circular recipient chromosome (thin lines; the 5′→3′ polarity is indicated by arrowheads), generating D-loops (step a). In panel B, only part of the recipient chromosome is shown. PriA catalyzes primosome assembly in the D-loops, and subsequent priming and replisome assembly lead to the formation of active replication forks (step b; newly synthesized DNA is indicated by stippled lines). The possible mechanisms of primosome and replisome assembly in the D-loop have been described previously (2, 25). The Holliday junctions are resolved (step c; only one of the four possible modes of resolution is shown). Completion of replication produces a recombinant (A) or repaired (B) chromosome (step d).

helicase loading, which is essential for the creation of a replication fork. The primosome subsequently primes lagging-strand synthesis by a DNA polymerase III replisome. The invading donor strand may be used to initiate leading-strand synthesis. The two oppositely oriented replication forks so assembled at the D-loops would replicate the remainder of the chromosome to yield a recombinant and a recipient chromosome (Fig. 1A). By exactly the same mechanism of PriA-dependent priming at the D-loop, the repair of DSBs would also be accomplished (Fig. 1B) (2, 36). The proposal is based on the following observations. (i) The SOS induction activates a novel mechanism for initiation of chromosome replication (iSDR) which can occur in the absence of normally required protein synthesis and transcription (for a review, see reference 3). (ii) The initiation depends on homologous-recombination functions (5). (iii) Under the conditions in which the nuclease activity of RecBCD (exonuclease V) is attenuated, artificially generated DSBs trigger a very similar mode of DNA replication, termed homologous-recombination-dependent DNA replication (2). (iv) iSDR cannot be induced in *priA*::*kan* mutants (25). In the work described in this report, we directly tested the above-described models by examining the effects of *priA*::*kan* mutations on homologous-recombination frequencies and by determining the sensitivity of *priA*::*kan* mutants to agents that cause DSBs.

MATERIALS AND METHODS

***E. coli* strains and plasmids.** The *E. coli* K-12 strains used are listed in Table 1. Two *priA* null mutant alleles, *priA1*::*kan* (18) and *priA2*::*kan* (29), were used to construct their derivatives by P1 transduction as described previously (25). pET-3c-K230R is a plasmid carrying the *priA300* allele, the expression of which is under the control of a T7 promoter (39). Perhaps via readthrough transcription of the *bla* gene of the vector, the plasmid-borne *priA300* gene is 10- to 20-fold overexpressed, even in cells which contain no T7 RNA polymerase (39). pHSG576 was previously described (38).

Media and growth conditions. Unless otherwise stated, cells were grown at 37°C with aeration by shaking. Growth was monitored by measuring cell numbers with a particle counter (Particle Data Inc., Elmhurst, Ill.). M9G is M9 salts-glucose medium (26) supplemented with required amino acids (50 μg/ml), thiamine (2 μg/ml), and thymine (8 μg/ml). CAA is M9G supplemented with Casamino Acids (0.2%; Difco Laboratories, Detroit, Mich.), required amino acids, thiamine, and thymine. LB is L broth (26) supplemented with 0.1% glucose.

Since *priA*::*kan* mutants are sensitive to rich media (25), cells were grown in M9G minimal medium in all experiments that involved *priA*::*kan* mutants. During overnight growth even in minimal medium, *priA*::*kan* mutant cultures accumulate a significant number of revertants, which can grow much faster than mutants. To avoid the accumulation of suppressor mutations, overnight cultures were prepared by spreading about 5×10^7 cells in a small area (about 15 cm^2) on an M9G plate and incubating the plate overnight. The next morning, cells were scraped from the plate, suspended in M9G, and inoculated into M9G, CAA, or LB to refresh the cells for experiments. *priA*$^+$ control cells were treated identically.

Determination of P1 transduction frequencies. To minimize potential bias in the transduction frequency, two phage P1 lysates were used. One lysate (P1.AQ7543) was prepared by growing phage in a mixture of seven strains which

TABLE 1. *E. coli* strains

Strain	Relevant genotype	Construction or reference
AQ634[a]	*priA*$^+$	30
AQ9215	AQ634 *priA1*::*kan*	AQ634 × P1.AQ8845; select Km^r
AQ9247	AQ634 *priA1*::*kan spa-47*	25
AQ9290	AQ634 *priA*$^+$/pET-3c-K230R	This work
AQ9293	AQ634 *priA1*::*kan*/pET-3c-K230R	This work
AQ9667	AQ634/pHSG576	This work
AQ9668	AQ634 *priA1*::*kan*/pHSG576	This work
AQ10572	AQ634 *priA2*::*kan*	AQ634 × P1.PN105; select Km^r
AB1157[b]	*priA*$^+$ *rec*$^+$ *sbc*$^+$	6
AQ9786	AB1157 *priA1*::*kan*	AB1157 × P1.AQ8845; select Km^r
AQ10082	AB1157 *priA2*::*kan*	AB1157 × P1.PN105; select Km^r
JC8679	AB1157 *recBC sbcA*	14
AQ9806	AB1157 *recBC sbcA priA1*::*kan*	JC8679 × P1.AQ8845; select Km^r
JC7623	AB1157 *recBC sbcB sbcC*	20
AQ10429	AB1157 *recBC sbcB sbcC priA1*::*kan*	JC7623 × P1.AQ8845; select Km^r
AQ10459	AB1157 *sfiA11*	AQ10452, spontaneous mutation to Ura$^+$ Km^s
AQ10477	AB1157 *sfiA11 priA1*::*kan*	AQ10459 × P1.AQ8845; select Km^r
AQ10479	AB1157 *sfiA11 priA2*::*kan*	AQ10459 × P1.PN105; select Km^r
CAG5053	HfrKL208 *zbe-280*::Tn*10*	34
CAG8209	HfrKL228 *zgh-3075*::Tn*10*	34
AQ8224	*recN1502*::Tn*5*	As RDK1540 (21)
AQ7543	*zzz*::Tn*10*	See Materials and Methods
AQ8845	*priA1*::*kan*/F′134	25
AQ9643	*asnA101*::*cat*	7
AQ10452	AB1157 *pyrD*::Tn*5 sfiA11*	Laboratory collection
PN105	*priA2*::*kan*	29

[a] The remaining genotype was F$^-$ *thyA his-29 trpA9605 pro ilv metB deoB* (or *deoC*).
[b] The remaining genotype was F$^-$ *argE3 his-4 leuB-6 proA2 thr-1 rpsL31 galK2 lacY1 ara-14 mtl-1 supE44.*

each carry a Tn*10* insertion between 31.0 and 39.5 min (34). The second lysate (P1.AQ9643) was grown in an *asnA101*::*cat* mutant (7). The advantage of using the *asnA*::*cat* marker is that there is another *asn* gene (*asnB*) in *E. coli* that can functionally substitute for *asnA*, reducing bias in the transduction frequency (10). Overnight cultures prepared as described above were inoculated in LB at a density of 5×10^7 cells per ml and grown to a density of 3×10^8 cells per ml. Cells were concentrated by centrifugation and suspended in M9G at 10^{10}/ml. A 100-μl aliquot of the cell suspension was mixed with 100 μl of phage P1 lysate at a multiplicity of infection of about 1 and incubated for 20 min in the presence of 2.5 μM $CaCl_2$ for phage adsorption. Cells were then washed twice with M9G and plated on M9G plates containing tetracycline (20 μg/ml) or chloramphenicol (50 μg/ml). Plates were incubated for 40 to 48 h before transductants were scored. Plating efficiencies of *priA*::*kan* mutant cells, which were typically in the range of 0.1 to 0.2 under the conditions used, were determined for each experiment, and transduction frequencies were corrected for viability before the relative transduction frequencies were calculated.

Determination of plating efficiencies of phage P1. Because of the sensitivity to rich media, plating efficiencies of phage P1 on *priA*::*kan* mutant strains could not be reproducibly determined by the standard procedure which utilizes LB (26). The procedure was modified as follows. Overnight cultures were refreshed in M9G to saturation (~2×10^9 cells per ml). A 100-μl aliquot of phage suspension (diluted with M9G containing 10 mM $MgSO_4$) was mixed with 200 μl of the saturated culture and incubated for 15 min. The mixture was then spread onto an M9G bottom agar plate, and this was overlaid with M9G soft agar containing 2.5 μM $CaCl_2$. The plate was incubated overnight. Under these conditions, the plating efficiency of phage on *priA*$^+$ strains was 5.7% ± 1.4% of the value determined by the standard procedure (see Table 3).

Hfr-mediated conjugation. Hfr and recipient cells were refreshed in CAA medium to 10^8/ml, mixed at a ratio of 1:10, and incubated for 60 min. Recombinants were selected for Tc^r and Sm^r for mating with AB1157 derivatives and for Tc^r and Cm^r for mating with AQ634 derivatives. For the purpose of counterselection, pHSG576 conferring Cm^r was introduced into AQ634 derivatives (Table 1). The recombination frequencies were corrected for viability of recipient cells.

Determination of sensitivities to gamma rays, mitomycin C, and UV light. Cells were grown in M9G to 2×10^8/ml, harvested by centrifugation, suspended in M9G buffer, and irradiated with gamma rays at a rate of 128 ± 8 rads/min. The source of gamma radiation was ^{137}Cs. The irradiated cells were plated on M9G plates after dilution. To determine sensitivity to mitomycin C, cells were grown to 2×10^8/ml, incubated with mitomycin C at a concentration of 1 μg/ml for up to 60 min, and plated on M9G plates after dilution. Sensitivity to UV light was determined as previously described (19).

RESULTS

***priA*::*kan* mutants are recombination deficient.** Two null alleles of the *priA* gene have been constructed in vitro by either replacing a part of the *priA* coding sequence with a *kan* gene (18) or simply inserting a *kan* gene fragment (29). These alleles were designated *priA1*::*kan* and *priA2*::*kan*, respectively (25). Both *priA* null mutations were found to significantly reduce the P1 transduction frequency. Thus, when normalized for the reduced viability of *priA* mutants (see Materials and Methods), the levels of P1 transduction were 20- to 50-fold lower than the wild-type level in two different genetic backgrounds (Table 2). Neither of the *priA* mutations drastically altered the P1 phage plating efficiency (Table 3), indicating that the mutations block neither the entry nor the replication of phage DNA. The *priA1*::*kan* mutation also caused a significant decrease in the frequency of recombinant formation after conjugation with Hfr strains, albeit less severely than it did in P1 transduction (Table 4). These results suggest that active PriA protein is required for homologous recombination.

At least in *priA2*::*kan* mutant cells, the *sfiA* (*sulA*) gene is chronically expressed and the constitutive synthesis of the SfiA protein, a cell division inhibitor, contributes to the decreased viability of the *priA*::*kan* mutant (29). Introduction of the *sfiA11* mutation, which inactivates the cell division inhibitor, elevated the plating efficiencies of *priA*::*kan* mutants to a range of 0.5 to 0.7 under the conditions used, and yet it failed to appreciably improve the P1 transduction frequency (Table 2). Therefore, even when *priA*::*kan* mutant cells had near-normal plating efficiencies, *priA*::*kan* mutations effectively reduced the P1 transduction frequency. This strongly supports the contention that the decreased viability of *priA*::*kan* mutants is not the cause of the observed reduction in P1 transduction.

The *priA300* allele encodes a mutant PriA protein (K230R)

TABLE 2. Relative frequencies of P1 transduction

Strain	Relevant genotype	Relative frequency[a]	
		With P1.AQ7543	With P1.AQ9643
AQ634	*priA*$^+$	1.00	1.00
AQ9215	*priA1*::*kan*	<0.0071	0.034
AQ10572	*priA2*::*kan*	0.019	0.015
AQ9290	*priA*$^+$/*priA300*	ND	0.35
AQ9293	*priA1*::*kan*/*priA300*	ND	0.49
AQ9247	*priA1*::*kan spa-47*	0.198	ND
AB1157	*priA*$^+$	1.00	1.00
AQ9786	*priA1*::*kan*	0.032	0.056
AQ10082	*priA2*::*kan*	0.036	0.022
AQ10459	*priA*$^+$ *sfiA11*	1.00	ND
AQ10477	*priA1*::*kan sfiA11*	0.052	ND
AQ10479	*priA2*::*kan sfiA11*	0.028	ND
JC8679	*priA*$^+$ *recBC sbcA*	1.00	ND
AQ9806	*priA1*::*kan recBC sbcA*	<0.015	ND
JC7623	*priA*$^+$ *recBC sbcBC*	1.00	ND
AQ10429	*priA1*::*kan recBC sbcBC*	0.058	ND

[a] Relative to the frequencies with *priA*$^+$ parental strains. ND, not determined. The standard error of the mean in these experiments is less than 25%.

which is capable of catalyzing the assembly of an active primosome but is completely deficient in the helicase, translocase, and ATPase activities associated with PriA (39). To see if the primosome assembly activity of PriA(K230R) is sufficient for homologous recombination, a plasmid which carries the *priA300* allele and which overexpresses the mutant PriA protein was introduced into a *priA1*::*kan* mutant. Introduction of the plasmid restored recombination proficiency to the *priA1*::*kan* mutant (Table 2). These results indicate that the primosome assembly function of PriA is sufficient for homologous recombination whereas the helicase, translocase, and ATPase activities are dispensable.

The *priA1*::*kan* mutation also inactivates the RecE and RecF pathways of homologous recombination. Activation of one of the two pathways of homologous recombination, the RecE or RecF pathway, suppresses the recombination deficiency of *recB* and *recC* mutants (11). The RecE pathway is activated by an *sbcA* mutation, and the RecF pathway is activated by mutations in the *sbcB* and *sbcC* genes. Thus, *recBC sbcA* and *recBC sbcB sbcC* mutants are recombination proficient. The *priA1*::*kan* mutation was found to decrease the P1 transduction frequency severely in the *recBC sbcA* mutant and moderately in the *recBC sbcB sbcC* mutant (Table 2). The *priA1*::*kan* mutation also significantly reduced conjugational recombination in the *recBC sbcA* mutant (Table 4). Therefore, PriA is required not only for the RecBCD pathway but also for the RecE and RecF pathways of homologous recombination.

TABLE 3. P1 phage plating efficiencies

Strain	Medium	Plating efficiency	
		No. of plaques/ml[a]	Relative efficiency[b]
AQ634	LB	$(1.74 \pm 0.55) \times 10^{10}$	17.6
AQ634	M9G	$(9.87 \pm 2.47) \times 10^{8}$	1.00
AQ9215	M9G	$(1.99 \pm 0.29) \times 10^{9}$	2.02
AQ10572	M9G	$(5.19 \pm 0.36) \times 10^{8}$	0.53

[a] Average (± standard error of the mean) of three independent determinations.

[b] Relative to the AQ634-M9G value.

TABLE 4. Relative frequencies of conjugational recombination

Strain	Relevant genotype	Relative frequency[a]	
		With HfrKL208	With HfrKL228
AQ9667	*priA*$^+$	1.00	1.00
AQ9668	*priA1*::*kan*	0.085	0.12
AB1157	*priA*$^+$	1.00	ND
AQ9786	*priA1*::*kan*	0.34	ND
JC8679	*priA*$^+$ *recBC sbcA*	1.00	ND
AQ9806	*priA1*::*kan recBC sbcA*	0.015	ND

[a] Relative to the frequencies with *priA*$^+$ parental strains.

***priA*::*kan* mutants are deficient in DSB repair.** Irradiation with gamma rays or incubation with mitomycin C causes DSBs in the chromosome which are lethal to the cell if not repaired. We compared the sensitivities of *priA*::*kan* mutants to these agents with those of a *recN* mutant which is known to be deficient in DSB repair (32). Figure 2 shows that both *priA1* and *priA2* mutants were hypersensitive to gamma radiation, and the severity of their hypersensitivity exceeded that of the *recN* mutant. Similarly, *priA1*::*kan* and *priA2*::*kan* mutants were found to be more sensitive to mitomycin C than the *recN* mutant (Fig. 3). Introduction of the *priA300*-carrying plasmid raised the mitomycin C resistance of *priA1*::*kan* mutants nearly to a wild-type level (Fig. 3). These results suggest that *priA*::*kan* mutants are deficient in DSB repair and that the function affected is primosome assembly.

***spa-47*, a suppressor mutation of *priA1*::*kan*, suppresses the defects in recombination and DSB repair.** *priA1*::*kan* mutants are sensitive to rich media. Thus, the plating efficiency of *priA1*::*kan* mutant cells is typically 10^{-3} to 10^{-4} on LB plates, whereas it is about 10^{-1} on minimal-medium plates (25). *priA2*::*kan* mutants showed similar plating efficiencies (data

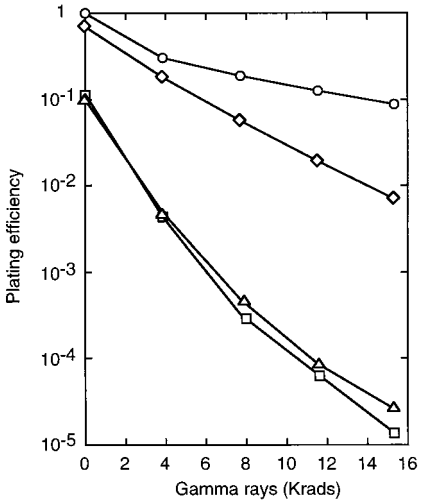

FIG. 2. Sensitivity of *priA* and *recN* mutants to gamma radiation. AB1157 (*priA*$^+$) (○), AQ9786 (*priA1*::*kan*) (△), AQ10082 (*priA2*::*kan*) (□), and AQ8224 (*recN1502*) (◇) were grown to densities of 2×10^8 cells per ml, and their sensitivities to gamma rays were determined as described in Materials and Methods.

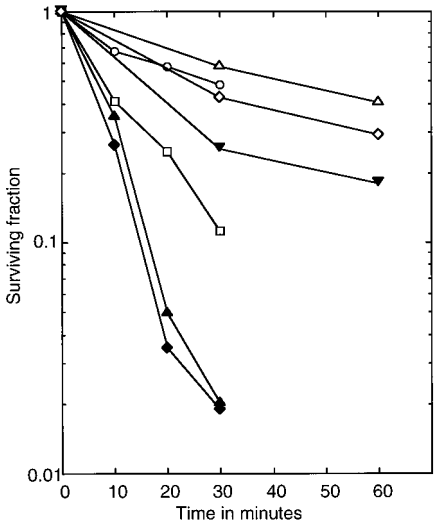

FIG. 3. Sensitivities of *priA* and *recN* mutants to mitomycin C. AB1157 (*priA*$^+$) (○), AQ634 (*priA*$^+$) (△), AQ9786 (*priA1*::*kan*) (▲), AQ10082 (*priA2*::*kan*) (◆), AQ8224 (*recN1502*) (□), AQ9290 (*priA*$^+$; pET-3c-K230R) (◇), and AQ9293 (*priA1*::*kan*; pET-3c-K230R) (▼) were grown to densities of 2×10^8 cells per ml, and their sensitivities to mitomycin C were determined as described in Materials and Methods.

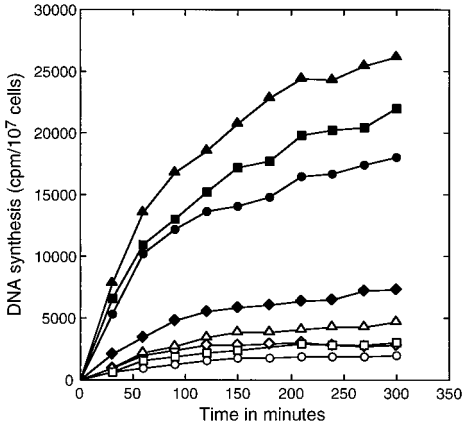

FIG. 5. Induction of iSDR in *priA1* mutant cells carrying a *priA300* plasmid. AQ634 (*priA*$^+$) (● and ○), AQ9215 (*priA1*::*kan*) (◆ and ◇), AQ9290 (*priA*$^+$; pET-3c-K230R) (▲ and △), and AQ9293 (*priA1*::*kan*; pET-3c-K230R) (■ and □) were grown to densities of 1.5×10^8 to 1.6×10^8 cells per ml and starved for thymine for 90 min (for AQ634, AQ9290, and AQ9293) or 120 min (for AQ9215). DNA synthesis in the presence of chloramphenicol was measured for thymine-starved cells (solid symbols) and unstarved control cells (open symbols) as described previously (22).

not shown). A revertant of *priA1*::*kan* capable of growing in rich media was previously isolated, and the suppressor mutation was designated *spa-47* (25). The *spa-47* mutation suppressed the hypersensitivity of *priA1*::*kan* mutants to UV radiation and mitomycin C (Fig. 4) and improved the ability of *priA1*::*kan* mutants to recombine in P1 transduction (Table 2).

Extragenic suppressor mutations which restore UV resistance to *priA2*::*kan* mutants were isolated by another group of investigators and were found to map to within the *dnaC* gene (32a). To see if *spa-47* also mapped within *dnaC* (99.0 min), AQ9247 (*priA1*::*kan spa-47*) was transduced to Tc^r with P1 phage grown on a strain carrying *zjj-202*::Tn*10*, which maps at 99.5 min (34). Of the Tc^r transductants tested, 67% (43 of 64) also inherited the rich-medium sensitivity. In a separate transduction experiment, the *zjj-202*::Tn*10* marker was found to be 85% linked to the *dnaC2*(Ts) mutation. It is very likely, therefore, that *spa-47* maps within or very near *dnaC*.

***priA300* allows induction of iSDR in *priA1*::*kan* mutants.** PriA is essential for initiation of iSDR (25). Thus, iSDR cannot be induced by thymine starvation in *priA1*::*kan* mutants (Fig. 5). Introduction of a plasmid carrying the *priA300* allele restored to *priA1*::*kan* mutant cells the ability to replicate DNA in the absence of protein synthesis and transcription after thymine starvation (Fig. 5). The effects of the vector plasmid as a control could not be examined because ColE1-type plasmids require a functional *priA* gene for replication (see the introduction). However, we previously demonstrated that a derivative of the pBR322 plasmid engineered to replicate in *priA* mutants did not restore iSDR inducibility (25). These results suggest that iSDR requires PriA-catalyzed primosome assembly. It is noteworthy that the introduction of the same plasmid into *priA*$^+$ cells enhanced iSDR activity after thymine starvation (Fig. 5), suggesting that the PriA-mediated priming activity is limiting in the wild-type cell.

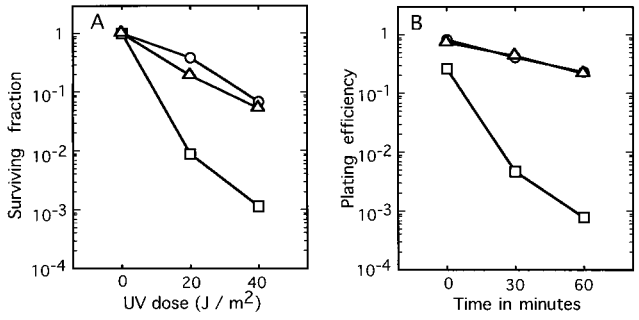

FIG. 4. Suppression of hypersensitivities of *priA1*::*kan* mutants to UV light and mitomycin C by *spa-47*. AQ634 (*priA*$^+$) (○), AQ9215 (*priA1*::*kan*) (□), and AQ9247 (*priA1*::*kan spa-47*) (△) were grown to densities of 2×10^8 cells per ml, and their sensitivities to UV light (A) and mitomycin C (B) were determined as described in Materials and Methods.

DISCUSSION

We have demonstrated that two null mutations of *priA* block P1 transduction in two strains with different genetic backgrounds. Successful transduction requires entry of the donor DNA fragment into the recipient cell followed by homologous recombination. The observation that the P1 phage produces plaques on *priA*::*kan* mutants at efficiencies similar to those of plaque production on the wild type indicates that DNA entry is not affected by the *priA*::*kan* mutations. This is corroborated by the fact that the *priA1*::*kan* mutation also affects recombinant formation after conjugation. We conclude that *priA*::*kan* mutations block some step in the homologous-recombination process.

iSDR, an SOS function inducible by DNA damage, is most likely initiated from a D-loop (5). The successful complementation of the defect of *priA* null mutants in iSDR by the *priA300* allele strongly suggests that the activity of PriA that is essential for iSDR initiation is the primosome assembly function. This supports the proposal that PriA catalyzes primosome assembly for DNA replication at the D-loop (25). Although efficient binding of PriA to single-strand DNA requires an n′-*pas* site, attempts to identify n′-*pas* in the *E. coli* chromosome have been unsuccessful (reviewed in reference 3). Evidence, however, suggests that PriA can interact with single-strand DNA without a canonical n′-*pas*, albeit with reduced efficiency (25).

Activation of iSDR requires SOS induction, perhaps for the generation of DSBs at the origins of replication (*oriM*s) and the attenuation of the nuclease activity of RecBCD (2). A similar DNA replication activity can be triggered by artificially generated DSBs without SOS induction, provided that RecBCD is attenuated by interaction with a chi site (2). The chi sequence is an octamer which, when encountered by a RecBCD enzyme, attenuates the nuclease activity, resulting in enhanced production of 3′ single-strand DNA ends for D-loop formation (for a review, see reference 28). Since the chi sequence can frequently be found in the *E. coli* chromosome (13), it is quite reasonable to expect that the ends of a linear DNA fragment brought in by P1 phage can trigger homologous-recombination-dependent DNA replication. A similar line of argument has led to the proposal that the ends generated by DSBs also initiate homologous-recombination-dependent DNA replication (2). The DNA replication initiated at the D-loops could complete the recombination and DSB repair processes as illustrated in Fig. 1. It should be noted that although the models for homologous recombination and DSB repair are shown separately in Fig. 1 for clarity, the two processes are identical. The only difference is the extent of replication required to complete the process.

The evidence obtained in this work provides strong support for the model described above. (i) iSDR, which is most likely initiated from D-loops, requires the primosome assembly function of PriA. (ii) The *priA*::*kan* mutations inhibit homologous recombination and DSB repair. (iii) The *priA300* allele complements the homologous-recombination and DSB repair defects of *priA*::*kan* mutants. (iv) The *spa-47* suppressor, which allows *priA*::*kan* mutants to undergo homologous-recombination-dependent DNA replication (25), restores the competence of homologous recombination and DSB repair to *priA*::*kan* mutants. (v) The *spa-47* mutation maps very near *dnaC*. The *dnaC* and *dnaT* genes constitute an operon (24), and both gene products are essential for the assembly of the ϕX174-type primosome (see the introduction). It is likely, therefore, that changes in the structure of the DnaC or DnaT protein caused by the mutation allow the assembly of active primosomes in the absence of PriA. DnaC and DnaT have previously been shown to be essential for iSDR (24). (vi) PriA is required not only for the RecBCD pathway but also for the RecE and RecF pathways of homologous recombination. It was previously shown that any one of the three recombination pathways can mediate initiation of iSDR (4, 5).

There exists solid evidence for the phage T4 system that shows the involvement of DNA replication in homologous recombination and vice versa (17, 27). On the other hand, previous reports addressing the possible role of extensive DNA replication in homologous recombination in *E. coli* are conflicting. The yield of recombinants after conjugation in a *dnaB*(Ts) recipient at the restrictive temperature was reported to be either greatly decreased (9) or stimulated (15). The recombination-by-replication model (Fig. 1A) predicts that donor DNA strands will always be joined to strands that are newly synthesized in recipient cells. Consistent with this prediction, density-labeling experiments which were designed to analyze the fate, in the recipient cells, of transferred DNA fragments after Hfr mating detected covalent joining of donor DNA strands to newly synthesized strands (33). No evidence which showed joining of donor DNA to the preexisting recipient strands was obtained. On the other hand, Oppenheim and Riley (31) detected recombinant molecules which contained both strands of the preexisting recipient DNA. Such molecules are not predicted by the model discussed above. They could be formed by double-strand replacement in a process which entails some type of breakage and reunion.

It is important to point out that the inhibition of homologous recombination by *priA*::*kan* mutations is not complete, suggesting that recombinants can also be produced by mechanisms (e.g., breakage and reunion) which can occur with no or very limited replication. This may explain at least in part the conflicting results regarding the involvement of DNA replication in homologous recombination. In fact, recombination by mechanisms other than recombination by replication would seem necessary to account for the multiple crosses within a given stretch of the chromosome which are regularly observed in P1 transduction and conjugation. Nevertheless, the evidence presented here strongly supports the notion that a large proportion of homologous-recombination events in *E. coli* involve extensive DNA replication.

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REFERENCES

1. **Allen, G. C., and A. Kornberg.** 1993. Assembly of the primosome of DNA replication in *Escherichia coli*. J. Biol. Chem. **268:**19204–19209.
2. **Asai, T., D. B. Bates, and T. Kogoma.** 1994. DNA replication triggered by double-stranded breaks in E. coli: dependence on homologous recombination functions. Cell **78:**1051–1061.
3. **Asai, T., and T. Kogoma.** 1994. D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. J. Bacteriol. **176:**1807–1812.
4. **Asai, T., and T. Kogoma.** 1994. The RecF pathway of homologous recombination can mediate the initiation of DNA damage-inducible replication of the *Escherichia coli* chromosome. J. Bacteriol. **176:**7113–7114.
5. **Asai, T., S. Sommer, A. Bailone, and T. Kogoma.** 1993. Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in *Escherichia coli*. EMBO J. **12:**3287–3295.
6. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:**525–557.
7. **Bates, D. B., T. Asai, Y. Cao, M. W. Chambers, G. W. Cadwell, E. Boye, and T. Kogoma.** 1995. The DNA box R4 in the minimal *oriC* is dispensable for initiation of *Escherichia coli* chromosome replication. Nucleic Acids Res. **23:**3119–3125.
8. **Bernstein, H., H. C. Byerly, F. A. Hopf, and R. E. Michod.** 1985. Genetic damage, mutation, and the evolution of sex. Science **229:**1277–1281.
9. **Bresler, S. E., V. A. Lanzov, and A. A. Lukjaniek-Blinkova.** 1968. On the mechanism of conjugation in *Escherichia coli* K12. Mol. Gen. Genet. **102:** 269–274.
10. **Cao, Y., and T. Kogoma.** 1995. The mechanism of *recA polA* lethality: suppression by RecA-independent recombination repair activated by the *lexA*-(Def) mutation in *Escherichia coli*. Genetics **139:**1483–1494.
11. **Clark, A. J.** 1991. *rec* genes and homologous recombination proteins in *Escherichia coli*. Biochimie **73:**523–532.
12. **Cox, M. M.** 1993. Relating biochemistry to biology: how the recombinational repair function of RecA protein is manifested in its molecular properties. Bioessays **15:**617–623.
13. **Faulds, D., N. Dower, M. M. Stahl, and F. W. Stahl.** 1979. Orientation-dependent recombination hotspot activity in bacteriophage lambda. J. Mol. Biol. **131:**681–695.
14. **Gillen, J. R., D. K. Willis, and A. J. Clark.** 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145:**521–532.
15. **Joshi, G. P., and O. Siddiqi.** 1968. Enzyme synthesis following conjugation and recombination in *Escherichia coli*. J. Mol. Biol. **32:**201–210.
16. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:**401–465.
17. **Kreuzer, K. N., and S. W. Morrical.** 1994. Initiation of DNA replication, p. 28–42. *In* J. D. Karam, J. W. Drake, K. N. Kreuzer, G. Mosig, D. H. Hall, F. A. Eiserling, L. W. Black, E. K. Spicer, E. Kutter, K. Carlson, and E. S. Miller (ed.), Molecular biology of bacteriophage T4. ASM Press, Washington, D.C.

18. **Lee, E. H., and A. Kornberg.** 1991. Replication deficiencies in *priA* mutants of *Escherichia coli* lacking the primosomal replication n′ protein. Proc. Natl. Acad. Sci. USA **88:**3029–3032.
19. **Lloyd, R. G., and S. D. Barbour.** 1974. The genetic location of the *sbcA* gene of *Escherichia coli*. Mol. Gen. Genet. **134:**157–171.
20. **Lovett, S. T., and A. J. Clark.** 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J. Bacteriol. **157:**190–196.
21. **Lovett, S. T., C. Luisi-DeLuca, and R. D. Kolodner.** 1988. The genetic dependence of recombination in *recD* mutants of *Escherichia coli*. Genetics **120:**37–45.
22. **Magee, T. R., and T. Kogoma.** 1990. Requirement of RecBC enzyme and an elevated level of activated RecA for induced stable DNA replication in *Escherichia coli*. J. Bacteriol. **172:**1834–1839.
23. **Marians, K. J.** 1992. Prokaryotic DNA replication. Annu. Rev. Biochem. **61:**673–719.
24. **Masai, H., and K. Arai.** 1988. Operon structure of *dnaT* and *dnaC* genes essential for normal and stable DNA replication of *Escherichia coli* chromosome. J. Biol. Chem. **263:**15083–15093.
25. **Masai, H., T. Asai, Y. Kubota, K. Arai, and T. Kogoma.** 1994. *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. EMBO J. **13:**5338–5346.
26. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Mosig, G.** 1994. Homologous recombination, p. 54–82. *In* J. D. Karam, J. W. Drake, K. N. Kreuzer, G. Mosig, D. H. Hall, F. A. Eiserling, L. W. Black, E. K. Spicer, E. Kutter, K. Carlson, and E. S. Miller (ed.), Molecular biology of bacteriophage T4. ASM Press, Washington, D.C.
28. **Myers, R. S., and F. W. Stahl.** 1994. χ and the RecBCD enzyme of *Escherichia coli*. Annu. Rev. Genet. **28:**49–70.
29. **Nurse, P., K. H. Zavitz, and K. J. Marians.** 1991. Inactivation of the *Escherichia coli* PriA DNA replication protein induces the SOS response. J. Bacteriol. **173:**6686–6693.
30. **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.
31. **Oppenheim, A. B., and M. Riley.** 1966. Molecular recombination following conjugation in *Escherichia coli*. J. Mol. Biol. **20:**331–357.
32. **Picksley, S. M., P. V. Attfield, and R. G. Lloyd.** 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional *recN* product. Mol. Gen. Genet. **195:**267–274.
32a. **Sandler, S. J., H. S. Samra, and A. J. Clark.** Genetics, in press.
33. **Siddiqi, O., and M. S. Fox.** 1973. Integration of donor DNA in bacterial conjugation. J. Mol. Biol. **77:**101–123.
34. **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.
35. **Smith, G. R.** 1988. Homologous recombination in procaryotes. Microbiol. Rev. **52:**1–28.
36. **Smith, G. R.** 1991. Conjugational recombination in E. coli: myths and mechanisms. Cell **64:**19–27.
37. **Smith, G. R., S. K. Amundsen, A. M. Chaudhury, K. C. Cheng, A. S. Ponticelli, C. M. Roberts, D. W. Schultz, and A. F. Taylor.** 1984. Roles of RecBC enzyme and Chi sites in homologous recombination. Cold Spring Harbor Symp. Quant. Biol. **49:**485–495.
38. **Takeshita, S., M. Sato, M. Toba, W. Masahashi, and T. Hashimoto-Gotoh.** 1987. High-copy-number and low-copy-number plasmid vectors for *lacZ* alpha-complementation and chloramphenicol- or kanamycin-resistance selection. Gene **61:**63–74.
39. **Zavitz, K. H., and K. J. Marians.** 1992. ATPase-deficient mutants of the *Escherichia coli* DNA replication protein PriA are capable of catalyzing the assembly of active primosomes. J. Biol. Chem. **267:**6933–6940.