Differential Suppression of *priA2::kan* Phenotypes in *Escherichia coli* K-12 by Mutations in *priA*, *lexA*, and *dnaC*

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Manuscript received July 24, 1995
Accepted for publication January 23, 1996

ABSTRACT

First identified as an essential component of the ϕX174 *in vitro* DNA replication system, PriA has ATPase, helicase, translocase, and primosome-assembly activities. *priA1::kan* strains of *Escherichia coli* are sensitive to UV irradiation, deficient in homologous recombination following transduction, and filamentous. *priA2::kan* strains have eightfold higher levels of uninduced SOS expression than wild type. We show that (1) *priA1::kan* strains have eightfold higher levels of uninduced SOS expression, (2) *priA2::kan* strains are UV^S and Rec^-, (3) *lexA3* suppresses the high basal levels of SOS expression of a *priA2::kan* strain, and (4) plasmid-encoded *priA300* (K230R), a mutant allele retaining only the primosome-assembly activity of $priA^+$, restores both UV^R and Rec^+ phenotypes to a *priA2::kan* strain. Finally, we have isolated 17 independent UV^R Rec^+ revertants of *priA2::kan* strains that carry extragenic suppressors. All 17 map in the C-terminal half of the *dnaC* gene. DnaC loads the DnaB helicase onto DNA as a prelude for primosome assembly and DNA replication. We conclude that *priA*'s primosome-assembly activity is essential for DNA repair and recombination and that the *dnaC* suppressor mutations allow these processes to occur in the absence of *priA*.

A protein called PriA was first identified and purified by its essential role in the ϕX174 *in vitro* primosome-assembly system (WICKNER and HURWITZ 1974; SHLOMAI and KORNBERG 1980). Helicase (3′ to 5′), translocase (3′ to 5′), ssDNA-dependent ATPase [this activity is dependent on a site called *pas* (see below)], and primosome-assembly activity have been defined for PriA (ZAVITZ and MARIANS 1992). The *priA* gene was identified using oligonucleotide probes synthesized to encode the amino terminal sequence of the purified protein (LEE *et al.* 1990; NURSE *et al.* 1990). Two mutations, *priA1::kan* and *priA2::kan* (see MATERIALS AND METHODS for their definitions) were constructed. These mutations caused filamentation and decreased cell viability (LEE and KORNBERG 1991; NURSE *et al.* 1991). In addition, *priA1::kan* caused UV sensitivity (LEE and KORNBERG 1991), sensitivity to rich medium, deficiency in homologous recombination following transduction, and inhibition of stable DNA replication (SDR) (MASAI *et al.* 1994). Although those phenotypes have not been demonstrated for *priA2::kan*, it has been shown that *priA2::kan* has eightfold higher levels of expression from *dinDp*, a *lexA*-repressed promoter (NURSE *et al.* 1991). The two different *priA* mutations both affected ϕX174 growth and plasmid replication but to different extents (LEE and KORNBERG 1991; NURSE *et al.* 1991). This may be because the *priA* mutants used in those tests were constructed in rich medium, and suppressor mutations may have occurred to optimize growth (MASAI *et al.* 1994). The addition of a *sulA* mutation to *priA2::kan* strains decreased the severity of the filamentation and cell inviability phenotypes (NURSE *et al.* 1991). A strain containing a suppressor of the rich medium sensitivity of *priA1::kan* was isolated. This suppressor, called *spa-47*, also suppresses *priA1::kan* as detected by restoration of recombination, DNA repair, and SDR (T. KOGOMA personal communication; MASAI *et al.* 1994). It is noteworthy that mutations in the *priA* gene have never been found in a screen for Rec^-, UV^S, or DNA replication mutants and that *priA* mutants show no major defect in DNA replication. However in *rnhA-339::cat dnaA46*^{ts} strains at 42°, *priA1::kan* causes cell death due to a defect in DNA replication (MASAI *et al.* 1994).

The first step in primosome assembly *in vitro* is PriA binding to ϕX174 ssDNA coated with SSB. Primosome assembly also requires PriB, PriC, DnaB, DnaC, DnaT proteins and a sequence or structure on the DNA called "*pas*" for *p*rimosome *a*ssembly *s*ite (reviewed in (ZAVITZ and MARIANS 1992; ALLEN and KORNBERG 1993). *pas*, first identified on the ϕX174 chromosome and since shown to be important in the replication regions of several plasmids (TANAKA *et al.* 1994), is recognized by the PriA protein. PriB binds the PriA-ssDNA complex and then, in the presence of PriC and DnaT, a complex of DnaB-DnaC loads DnaB on to the ssDNA (ALLEN and KORNBERG 1993). The complex of PriA, PriB and DnaB is called the preprimosome, and upon the addition of primase, DnaG protein, the complex primes DNA synthesis by Pol III holoenzyme.

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PriA's role in recombination may be the formation of a DNA replication fork via primosome assembly at or near a D-loop or Holliday structure (ASAI and KOGOMA 1994; MASAI *et al.* 1994). This model assumes that there are either yet undiscovered *pas* sites on the *Escherichia coli* chromosome available for PriA to recognize or that some *pas*-independent mechanism exists for PriA binding (MASAI *et al.* 1994). We tested this model in two ways. First, we asked if the primosome-assembly activity of PriA was necessary for recombination or if the helicase, translocase, *pas*-specific ssDNA-dependent ATPase activity of PriA was needed. Second, we enquired if other replication proteins, particularly those involved in primosome assembly, could be implicated in the recombinational repair process. The former was investigated by looking at the ability of a *priA300* mutant whose mutant PriA300 protein retains only primosome-assembly activity (ZAVITZ and MARIANS 1992) to complement the Rec$^-$ and UV^S phenotypes of *priA2::kan*. The second possibility was explored by looking for indirect suppressors of *priA2::kan*. We show that the primosome-assembly activity of *priA300* can complement *priA2::kan* and that mutations in *dnaC*, whose role is to regulate activity and specificity of loading DnaB helicase, can indirectly suppress *priA2::kan*. In both cases *priA2::kan* strains become phenotypically Rec$^+$ and UV^R.

MATERIALS AND METHODS

Nomenclature: Two genetically engineered mutations of *priA* have been made and transferred to the chromosome: *priA*$^-$ (LEE and KORNBERG 1991) and *priA::Kan*^{r} (NURSE *et al.* 1991). To better distinguish between these two mutations, KOGOMA and his colleagues have renamed them *priA1::kan* and *priA2::kan*, respectively (MASAI *et al.* 1994). The *priA1::kan* allele (LEE and KORNBERG 1991) is a partial deletion of the middle of the *priA* gene with the concomitant insertion of the kanamycin-resistance gene. The *priA2::kan* allele (NURSE *et al.* 1991) is a simple insertion of the kanamycin-resistance gene at codon 153. The new *priA* and *dnaC* allele numbers presented in this paper have been designated by the *E. coli* Stock Center.

Bacterial strains and plasmids: All bacterial strains used in this work are derivatives of *E. coli* K-12 and are described in Table 1. Strains for all experiments described were grown in 56/2 minimal medium (WILLETTS *et al.* 1969) at 37°. The protocol for P1 transduction has been described elsewhere (WILLETTS *et al.* 1969). The *priA2::kan* mutation was always the last mutation transduced into a strain because this mutation decreases the inheritance of genetic markers. In addition, due to *priA1::kan* strain's sensitivity to rich medium (ASAI and KOGOMA 1994), all transductions selecting for *priA2::kan* were done on minimal medium (WILLETTS *et al.* 1969). pET-3c-Y1 and pET-3c-K230R are plasmids that contain *priA*$^+$ and *priA300*, respectively (ZAVITZ and MARIANS 1992).

UV induction of *sulAp-lacZ*: Dervatives of DM4000 [*dnaC*$^+$ *priA*$^+$ *sulA*::Mu-d(Ap, *lac*, *B*::Tn*9*)] were grown in minimal medium at 37° until the cells reached log phase. One milliliter of unirradiated cell culture was used as the 0-min sample. Five milliliters were then irradiated at 0.5 joules/m^2/sec for 10 sec. Four milliliters of the UV-irradiated culture were transferred to a clean flask containing 9 ml of fresh medium, which was then incubated at 37° and aerated by shaking in a darkened room. Samples were removed at indicated times and assayed for β-galactosidase (SANDLER and CLARK 1993). Since β-galactosidase is produced only from the *sulA::lacZ* transcriptional fusion gene contained in this strain, the amount of β-galactosidase reflects *sulAp* expression. The results shown are averages of three experiments with each strain.

To be sure that JC19102 (*priA2::kan lexA3*) had not lost the *sulAp-lacZ* reporter gene during the strain construction, we performed two controls. First, several independent transductants from the cross in which JC19102 was made were tested for β-galactosidase levels and gave similar results to JC19102 (data not shown). Second, *lexA*::Tn*5* was transduced from JC13199 into JC19098 to be sure that the *sulAp-lacZ* reporter gene was not lost while making JC19098. The resulting strain, JC19108, showed 12,000 units of β-galactosidase, suggesting that the *sulAp-lacZ* gene was intact.

UV survival tests: Strains were incubated at 37° in minimal medium until they were in log phase and then were irradiated for various amounts of time at a rate of 0.5 joule/m^2/sec and appropriately diluted into 56/2 buffer. Appropriate dilutions (0.1 ml) were spread on minimal medium agar plates. Cells were incubated at 37°, and colonies were counted after 48–72 hr. All steps after the irradiation took place in a darkened room. The results shown are averages of two or three experiments with each strain unless otherwise indicated. Survival is relative to unirradiated cultures.

Selection and identification of the *dnaC* mutants: A culture of JC18983 was grown in minimal medium until the cells were in log phase. The cells were irradiated with 20 joules/m^2 of UV light to mutagenize the DNA. About 10^5–10^7 total cells (10^3–10^5 viable cells) were then spread per plate (a typical experiment was 10 plates) on minimal or rich medium plates and incubated at 37° for 24–48 hr in the dark. Large surviving colonies that distinguished themselves in some cases against a nearly confluent background were found at a frequency of $\sim 10^{-6}$–10^{-7} (total cells). These were tested for their sensitivity to UV irradiation. Several independent UV^R strains were found, saved, and studied further. Conjugation with an Hfr strain was done as previously described (WILLETTS *et al.* 1969) and was used to map the reversion in JC19008 to the *dnaC* region. Other reversions were mapped by P1 transduction to the *dnaC* region. All were further analyzed at the molecular level by DNA sequencing. DNA sequencing of the wild-type and mutant *dnaC* and *dnaT* genes were done using ssDNA generated by asymmetric PCR. Asymmetric PCR was done according to an established protocol (SHYAMALA and AMES 1989) with slight modifications. Sequences of specific primers used are available on request.

Recombinant frequencies: Measurement of recombinant frequencies by transduction and conjugation require transfer of DNA to a recipient as well as recombination. To determine transductional transfer frequency to a putative Rec$^-$ strain, a standard method is to monitor the efficiency of plating (eop) of the transducing phage P1. We used this method and corrected transductant frequencies appropriately.

RESULTS

***priA2::kan* mutants are sensitive to UV irradiation and are recombination deficient:** Phenotypes for the *priA1::kan* deletion/insertion mutant include UV sensitivity, recombination deficiency, and filamentation (LEE and KORNBERG 1991; MASAI *et al.* 1994). Since it is possible that insertion mutations at different points in a gene for a multifunctional protein could result in

TABLE 1

***E. coli* strains used in this work**

Strain no.	*priA*	*dnaC*	Other relevant genes	Source or Reference[a]
AQ9215	*1*	+		T. KOGOMA
CAG18430	+	+	*zjj-202::*Tn*10*	SINGER *et al.* (1989)
DM4000[b]	+	+		M. VOLKERT
JC13199	+	+	*lexA::*Tn*5*	Laboratory strain
JC13509[c]	+	+	*sulB103 his-4*	Laboratory strain
JC13519[c]	+	+	*lexA3 malE::*Tn*10*	Laboratory strain
JC18983	*2*	+		P1 · PN105 → DM4000[d]
JC19008	*2*	*809*		This work
JC19009	*2*	*810*		This work
JC19018	*2*	*809*	*zjj-202::*Tn*10*	P1 · CAG18430 → JC19008[e]
JC19021	*2*	+	*sulB103 his-4*	P1 · PN105 → JC13509[d]
JC19098	+	+	*lexA3 malE::*Tn*10*	P1 · JC13519 → DM4000
JC19102	*2*	+	*lexA3 malE::*Tn*10*	P1 · PN105 → DM4000[d]
JC19123	*2*	*811*		This work
JC19124	*2*	*812*		This work
JC19125	*2*	*813*		This work
JC19126	*2*	*814*		This work
JC19135	*2*	*815*		This work
JC19136	*2*	*816*		This work
JC19137	*2*	*817*		This work
JC19138	*2*	*818*		This work
JC19141	*2*	*819*		This work
JC19108	+	+	*lexA::*Tn*5*	P1 · JC13199 → JC19098[d]
JC19197	*1*	+		P1 · AQ9215 → DM4000[d]
PN105	*2*	+		NURSE, *et al.* (1991)

[a] Transductions are described symbolically as follows: P1 · PN105 → DM4000 means DM4000 was transduced by a P1 phage lysate made on host PN105.

[b] Δ*(lac-pro)XIII hisG4 argE3 thr-1 ara-14 xyl-5 mtl-1 rpsL31 sulA::*Mu-d(Ap, *lac, B::*Tn*9*). Note however that this strain appears Thr$^+$ on testing (data not shown).

[c] A derivative of SK362 (ZIEG and KUSHNER 1977).

[d] Select kanamycin resistance.

[e] Select tetracycline resistance and screen for UV^R.

different phenotypes, we tested whether or not the UV-sensitivity and recombination-deficiency phenotypes described for the *priA1::kan* strain were the same for the *priA2::kan* strain. Table 2 shows that the *priA2::kan* mutation causes a decreased inheritance of markers by P1 transduction of ~50-fold compared to wild type. Figure 1 shows that *priA2::kan* causes a 50-fold decrease in UV resistance relative to wild type. We note that the slopes for survival between the *priA*$^+$ and *priA2::kan* strains are equal after an initial 50-fold drop. The significance of this remains to be determined. We conclude that *priA2::kan* and *priA1::kan* cause similar phenotypes. It is therefore probable that these phenotypes result from lack of a wild-type *priA* gene product rather than the activity of a partially functional truncated PriA protein.

Constitutive and UV-inducible *sulAp-lacZ* expression in *priA1::kan* and *priA2::kan* mutant strains: The *priA1::kan* and *priA2::kan* mutations cause cells to filament (LEE and KORNBERG 1991; NURSE *et al.* 1991). This phenotype suggests that, in a *priA* null mutant, *lexA*-repressed gene expression (SOS expression) may be higher in the absence of a DNA-damaging agent than in wild type. In confirmation, NURSE *et al.* (1991) showed that *priA2::kan* provoked an eightfold higher level of expression of a reporter gene, *lacZ*, fused to *lexA* (SOS) regulon promoter, *dinDp*. They speculated that *priA2::kan* might lead to a less stable replication fork, which in turn might cause defects in the way in which ssDNA is managed during lagging strand DNA synthesis. LEE and KORNBERG (1991), however, reported normal levels of LexA protein in a *priA1::kan* strain and concluded that the filamentation phenotype was not due to RecA-protein-promoted LexA cleavage. We have tested constitutive *lexA* regulon expression in both *priA* mutant strains by using *lacZ* as a reporter gene fused to the *lexA* regulon promoter, *sulAp*. Both strains showed the same amount of constitutive *lacZ* expression (Figure 2). As suggested by MASAI *et al.* (1994), LEE and KORNBERG (1991) may have not seen reduced LexA protein levels in the *priA1::kan* strain because the strain had acquired a spontaneous suppressor mutation.

We also tested UV induction of *sulAp-lacZ* in both

TABLE 2

Relative frequencies of inheritance of genetic markers by P1 transduction

Strain	Plasmid	*priA*	*dnaC*	*lexA*	Relative frequency
DM4000		+	+	+	1.00[a]
JC18983		*2*	+	+	0.02[a]
JC19008		*2*	*809*	+	0.50[a]
JC19098		+	+	*3*	0.43[a]
JC19102		*2*	+	*3*	0.014[a]
JC19021	pET3c-Y1	+/*2*[c]	+	+	1.00[b]
JC19021	pET3c-K230R	*300/2*[c]	+	+	0.52[b]

[a] Selection is for His$^+$ and relative to DM4000. The absolute frequency of inheritance of His$^+$ transductant per P1 for DM4000 is 1.3×10^{-5}. Numbers show average of three experiments. The numbers have been normalized for the efficiency of plating P1 on the strain to wild type. The plating efficiency of P1 on DM4000, JC19008, and JC19098 were equivalent. The efficiencies of JC19893 and JC19102 were down about twofold from the others. The efficiency of plating was determined by growing the cells in minimal media and using the protocol in WILLETS *et al.* (1969).

[b] Selection is for His$^+$ and relative to pET3c-Y1/JC19021 strain. Number shows the average of two experiments.

[c] The two alleles indicate that these strains are heterozygous for *priA*. The first allele listed is on the plasmid and the second allele listed is on the chromosome.

priA1::kan and *priA2::kan* strains (Figure 2). Both strains show approximately the same pattern of induction. The differences in onset and maxiumal levels of induced expression may be due to differences in the mutations or to experimental varibility.

We note that after wild type has reached its maximum level of β-galactosidase, the levels of β-galactosidase in both *priA* mutant strains continue to increase slightly. The fact that the β-galactosidase levels in *priA* mutant strains do not reach a maximum and then decrease like wild type could be due to a slower rate of LexA synthesis, greater stability of β-galactosidase, longer persistence of DNA damage, or differential ability of the strains to survive irradiation and grow. We conclude that *priA1::kan* and *priA2::kan* mutants have higher basal levels of, and are UV-inducible for, *sulAp-lacZ* expression. We presume that this is due to derepression of the *lexA* regulon.

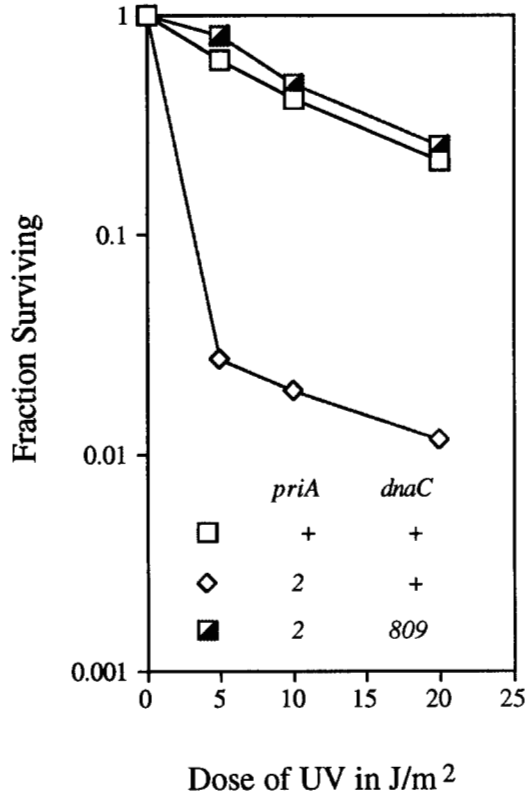

FIGURE 1.—Survival after UV irradiation of strains: *priA*$^+$ *dnaC*$^+$ (DM4000), *priA2::kan dnaC*$^+$ (JC18983) and *priA2::kan dnaC809* (JC19008). Results show the average of two experiments.

lexA3* suppresses the high basal levels of SOS expression caused by *priA2::kan: If the high basal level of *sulAp-lacZ* expression in a *priA2::kan* strain is due to derepression of the *lexA* regulon, then introducing *lexA3*, an uninducible allele of *lexA* that is deficient in RecA-mediated autoproteolysis (LITTLE *et al.* 1980), into that strain should suppress the high basal level of *sulAp-lacZ* expression. We see that *lexA3* decreases the levels of a *priA2::kan* mutant ~20-fold from 710 units (JC18983) to 40 units (JC19102) (Figure 2 and data not shown). This level is threefold below that of wild type and equal to levels of a strain with *lexA3* alone (JC19098). Hence, we conclude that the high basal level of β-galactosidase in *priA2::kan* strains is due to derepression of the *lexA* regulon.

If the Rec$^-$ and UV^S phenotypes of a *priA2::kan* strain resulted from an increased level of SOS expression, then *lexA3* should also suppress these phenotypes. Table 2 shows that *lexA3* has no effect on *priA2::kan*'s ability to reduce inheritance. It was not possible to determine whether or not *lexA3* also suppresses the UV sensitivity of *priA2::kan* because *lexA3* itself causes UV sensitivity (MOUNT *et al.* 1972). We conclude that while *lexA3* is able to suppress the high basal levels of β-galac-

TABLE 3

dnaC* suppressors of *priA2::kan

Strain	*dnaC*	Codon in *dnaC*	Change	Independent clones
JC19008[a]	*809*	176	GAA (glu) to GGA (gly)	2
JC19009[a]	*810*	176	GAA (glu) to GGT (gly)	2
JC19123[a]	*811*	184	ATC (ile) to TTC (phe)	2
JC19124[a]	*812*	185	AAC (asn) to CAC (his)	3
JC19125[a]	*813*	201	CTG (leu) to ATG (met)	1
JC19126[a]	*814*	144	GAT (asp) to GCT (ala)	1
JC19135[b]	*815*	188	GTC (val) to ATC (ile)	1
JC19136[b]	*816*	189	GAT (asp) to AAT (asn)	2
JC19137[b]	*817*	184	ATC (ile) to ATG (met)	1
JC19138[b]	*818*	168	ATC (ile) to ATG (met)	1
JC19141[b,c]	*819*	122	GAG (glu) to GCG (ala)	1
		135	GTG (val) to TTG (leu)	

[a] Selected as UV^R on minimal media using 20 J/m^2. See MATERIALS AND METHODS.
[b] Selected as UV^R on Luria Broth using 20 J/m^2. Cells were grown in minimal medium until in log phase, treated with UV light and then plated on Luria plates at 37°. See MATERIALS AND METHODS.
[c] This strain is a double mutant.

tosidase seen in *priA2::kan* strains, it is not able to suppress the defect in recombination caused by *priA2::kan* (Table 2). Thus it is likely that the *priA2::kan* strain's defect in recombination does not result solely from high level expression of *lexA*-repressed genes.

***priA300* restores UV resistance and recombination proficiency to a *priA2::kan* mutant:** The *priA* protein has helicase, translocase, *pas*-specific ssDNA-dependent ATPase and primosome-assembly activities. Others have constructed a mutant of *priA*, *priA300*, that has an arginine instead of a lysine at position 230 in the putative phosphate-binding hole of the protein (ZAVITZ and MARIANS 1992). Biochemical analysis of the mutant PriA300 protein has shown that this mutant protein is deficient in ATPase, translocase and helicase activity. The PriA300 protein however retains the ability to associate with *pas* and assemble a primosome (ZAVITZ and MARIANS 1992).

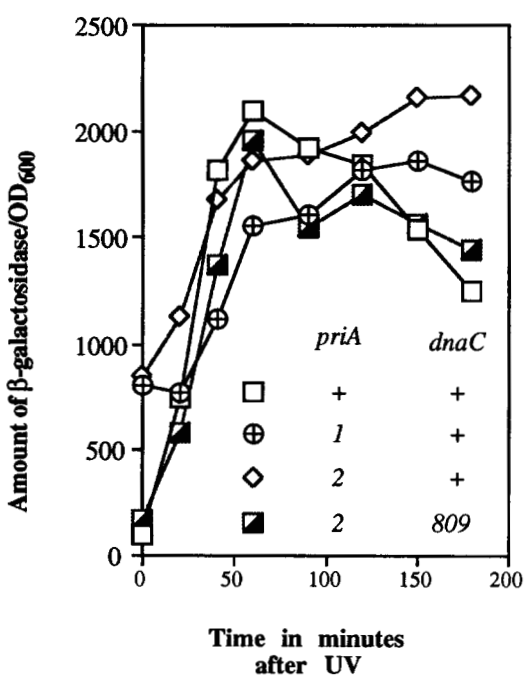

FIGURE 2.—UV-inducible β-galactosidase expression from strains: *priA*$^+$ *dnaC*$^+$ (DM4000), *priA1::kan dnaC*$^+$ (JC19197), *priA2::kan dnaC*$^+$ (JC18983) and *priA2::kan dnaC809* (JC19008). Results show the average of three experiments. Strains were irradiated with 5 J/m^2.

Since many recombination proteins have helicase activity [*e.g.*, RecBCD, RecQ, RuvAB (reviewed in LLOYD and SHARPLES 1992)], we wanted to test whether or not the helicase activity of PriA was necessary for its role in recombination or if some other activity of PriA is needed. If the helicase activity of PriA is necessary for recombination and DNA repair, then *priA300* should not be able to restore the UV resistance and recombination proficiency to a *priA2::kan* mutant. On the other hand, if *priA300* does complement *priA2::kan*, then we could conclude that the helicase, translocase, and ATPase activities of PriA are not needed for these processes and that primosome assembly is most likely the necessary activity for recombination and DNA repair.

To test this hypothesis, we transformed JC19021 containing *priA2::kan* on the chromosome with plasmids that contained either *priA*$^+$ or *priA300*. The fact that the *priA300* plasmid could transform a *priA2::kan* strain and be stably maintained is evidence that the primosome-assembly function of *priA300* is functional *in vivo* (MASAI *et al.* 1994). The inability of *priA2::kan* strains to be transformed by derivatives of ColE1 made it impossible to test the complementation of a vector plasmid without a *priA* allele. Table 2 shows that a *priA2::kan* strain with the *priA*$^+$ and *priA300* plasmids have about equal frequencies of P1-mediated inheritance. *priA*$^+$ and *priA300* complement the UV sensitivity of

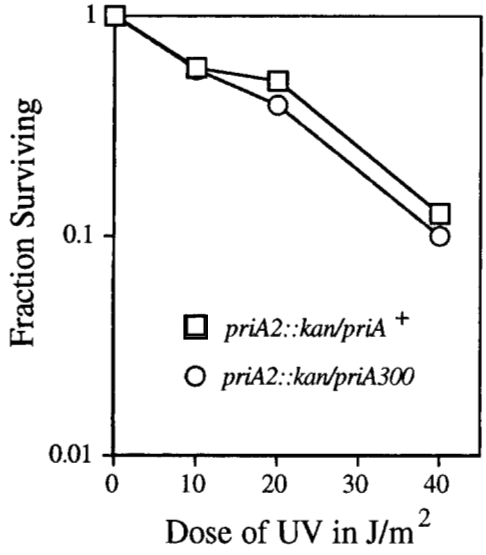

FIGURE 3.—Survival to UV irradiation of strains derived from JC19021 strains carrying plasmids with either *priA*+ or *priA300*. Results shown are from a single experiment. Similar results were obtained in an independent qualitative replica plate patch test.

priA2::kan strains nearly identically; both bring the resistance up to wild-type levels (Figure 3).

It is possible that the plasmid-encoded *priA300* gene could have recombined with the *priA2::kan* gene on the chromosome to produce a wild-type *priA* gene, thus providing the UV resistance and recombination proficiency we observed in what we thought was a *priA2::kan/priA300* meroploid strain. To test this possibility, we performed two experiments. First, we confirmed that the putative *priA2::kan/priA300* meroploid strain was still resistant to kanamycin; hence the chromosomal copy of *priA* was still mutant. Second, plasmid DNA was isolated from the merodiploid strain and restricted with *Bsi*WI endonuclease. The presence of this restriction enzyme recognition sequence in the mutant *priA* gene is diagnostic of the *priA300* mutation (ZAVITZ and MARIANS 1992). Treatment of plasmid DNA with this enzyme results in a shift of the DNA gel mobility with no uncleaved plasmid DNA apparent (data not shown). Given that we could detect 0.5% uncut plasmid DNA (data not shown) and that ColE1 derivatives have a copy number per chromosome of ~15 (TWIGG and SHERRATT 1980), <7% of the cells could be harboring a single plasmid with a wild-type copy of the gene. Hence, if the Rec^+ and UV^R phenotypes were due to a single copy of $priA^+$ generated by homologous recombination, only 7% of the cells should be Rec^+ and UV^R. Since 50% of the cells appear to be Rec^+ and 100% of the cells to be UV^R, we think these data are consistent with the nature of the strain being a heterozygous meroploid.

Two explanations of the Rec^+ UV^R phenotype of the merodiploid are possible. First, reestablishment of the repression of the LexA regulon might be responsible since the *priA300* gene on a multicopy plasmid reverses the filamentation phenotype of *priA2::kan* (ZAVITZ and MARIANS 1992). Because *lexA3,* which reestablished LexA regulon repression, did not make the phenotype Rec^+ (Table 2), we do not accept this explanation. Thus we prefer the second explanation, that the activities remaining in PriA300 protein, when expressed from a multicopy plasmid, are sufficient to perform $PriA^+$ protein's role in recombination and DNA repair. We hypothesize that PriA's primosome-assembly activity, or some other yet unknown activity of PriA that is not affected by the *priA300* mutation, is crucial for PriA's role in recombination and DNA repair.

Chromosomal mutations that indirectly suppress the UV sensitivity, recombination deficiency, and high levels of SOS expression of *priA2::kan* mutations: From the data presented above, it appears that the primosome-assembly activity of PriA is crucial for DNA repair and recombination. One way to test this idea further is to isolate and study indirect suppressors of *priA2::kan.* If the above is true, then one might expect that indirect suppressors of the UV sensitivity of *priA2::kan* would map in other primosome-assembly genes.

To isolate indirect suppressors of the UV-sensitive phenotype of *priA2::kan* strains, we UV-irradiated a culture of JC18983, plated the cells, and scored survivors for their UV sensitivity using a qualitative replica plating patch test. In independent experiments, several UV^R clones were found and further analyzed. Two from one experiment called JC19008 and JC19009 were shown still to carry *priA2::kan* by transduction backcrosses (data not shown). A detailed analysis of JC19008 is described below.

JC19008 was tested quantitatively for its UV sensitivity, recombination proficiency and ability to show the SOS response. Figure 1 shows that the resistance of JC19008 to UV irradiation is 50-fold greater than that of the *priA2::kan* progenitor strain and similar to that of the wild-type strain. Recombination proficiency as measured by P1 transduction is also restored in JC19008 (Table 2). Figure 2 shows that JC19008 has normal basal levels of *sulAp-lacZ* expression and that the pattern of expression after UV irradiation is more or less equivalent to wild type. We conclude that the suppressor of UV sensitivity in this *priA2::kan* strain also suppresses two other phenotypes of *priA2::kan:* recombination deficiency and the high basal level of SOS expression.

The *priA2::kan* indirect suppressor mutations map in *dnaC:* Since the *priA2::kan* strain carrying the suppressor, JC19008, was Rec^+, standard genetic strategies for mapping the suppressor mutation(s) could be employed. We first mapped the suppressor(s) in JC19008 by *Hfr* crosses (SINGER *et al.* 1989). We monitored the presence of the incoming wild-type allele by the reversal of two phenotypes: light blue to dark blue colonies on plates containing X-gal (20 μg/ml) and the change from UV^R to UV^S. Mapping results were consistent with the mutation lying in the 20-min region of the chromo-

some between 90 and 10 min (data not shown). Several genes known to be involved with DNA replication map in this region. We tested the hypothesis that the mutation mapped in the *dnaTC* region at 99 min. To do this we transduced JC19008 with a P1 lysate made from CAG18430. This strain has a Tn*10* insertion mutation, *zjj-202*::Tn*10*, at 99.5 min (SINGER *et al.* 1989). Twelve of 24 Tet^R transductants tested also acquired UV sensitivity. We concluded that at least one mutation in JC19008 responsible for the suppression of the UV^S and Rec^- phenotypes of JC18983 mapped within a region that was cotransducible with *zjj-202*::Tn*10*.

It is possible that the mutation that is cotransducible with *zjj-202*::Tn*10* is not sufficient for the suppression of the UV-sensitive phenotype of *priA2::kan* and that other mutation(s) elsewhere in the chromosome also contribute to the suppression. To test this possibility, a P1 lysate was made from JC19018, a Tet^R UV^R Rec^+ transductant of the CAG18430 transduction cross with JC19008 described above. This P1 lysate was used to transduce the nonsuppressed *priA2::kan* strain, JC18983, to Tet^R. We saw a ~100-fold increase in the number of Tet^R transductants (P1 lysate from JC19018), relative to His^+ transductants (P1 lysate from CAG18430) (data not shown). This is consistent with the transducing particles carrying a dominant Rec^+ (suppressor) allele. 94/96 Tet^R transductants in this cross had concomitantly acquired UV^R. We conclude that only a single mutation or multiple closely linked mutations are sufficient to suppress the UV^S phenotype of *priA2::kan* strains. If the transducing particles do express a dominant suppressing allele to form a stable recombinant of the *priA2::kan* strain, then it is possible that the 2/96 Tet^R transductants that did not inherit UV^R are examples of a crossover between the Tn*10* transposon and the suppressor allele.

The suppressor mutation mapped near the *dnaT* and *dnaC* genes. Since both of these genes interact with *priA* in ϕX174 primosome assembly, we hypothesized that the mutation causing the suppression would lie in one of them. We tested this hypothesis directly by sequencing a single strand of each of these two genes directly from the mutant and wild-type chromosomes. We found only one deviation from the wild-type sequence in either the *dnaT* or *dnaC* genes of JC19008. This difference was a change at codon 176, a GAA (glu) to a GGA (gly) in the *dnaC* gene (Table 3). We therefore have named this mutation *dnaC809*.

To determine whether or not mutations in other genes would suppress *priA2::kan*, we analyzed 16 other independent UV^R revertants of JC18983. We found that, like JC19008, the suppressors in all these strains (Table 3) were cotransducible with *zjj-202*::Tn*10*. The DNA sequence of the *dnaC* gene in all these strains was determined in the region of codon 176, the site of the *dnaC809* mutations. In the mutants tested, all had a mutation that changed a codon between codons 122 and 201 of *dnaC*. Table 3 and Figure 4 show that we found a total of 10 mutants with different single codon changes and one mutant with a double codon change. Since we found five cases in which independent mutants have the same sequence change (Table 3), it is likely that we have most examples of the type of mutations that can suppress the UV^S phenotype of a *priA2::kan* strain. We conclude that all independent mutants found both restore the UV resistance to a *priA2::kan* strain and have changes in the carboxy terminal half of the *dnaC* gene. We tentatively conclude that these mutations produce the suppression observed, although we have not ruled out rigorously that some other mutations exist in these strains that are closely linked with the *dnaC* mutations and produce the suppression. We therefore have no evidence that mutations in genes other than *dnaC*, with the exception of *priA300*, will suppress *priA2::kan* and restore UV resistance and recombination proficiency.

DISCUSSION

Information concerning the coordination between DNA replication and recombination/DNA repair in *E. coli* has been elusive. A reason for this may be that genes involved with DNA replication are essential for cell growth so that mutations in these genes have been difficult to test for recombination deficiency. *priA* is the exception. Biochemistry has pointed to a role for it in DNA replication. Viability of *priA* mutants has permitted the detection of their UV sensitivity and recombination deficiency. Thus replication and recombination appear to be coordinated through the action of the PriA protein. We have elaborated this hypothesis by showing the following: (1) the primosome assembly activity encoded by *priA* is most likely the activity that is important for recombination, and (2) another DNA replication protein, DnaC, is implicated in the recombination process.

The necessity of PriA's primosome-assembly function in recombination is supported by the experiments showing that *priA300* can complement the UV^S and Rec^- phenotypes of *priA2::kan*. *In vitro* PriA300 possesses only the primosome-assembly activity of PriA and not the helicase, ATPase or translocase activities. It is still possible, however, that *priA300* may complement only because it is overexpressed from a multicopy vector or that *priA300* may have some other activity that is needed for recombination. The *in vivo* roles of the helicase, translocase, and *pas*-specific ssDNA-dependent ATPase activities remain to be elucidated.

If *priA* participates in recombination by initiating primosome assembly, mutations in other assembly proteins may be able to compensate for PriA's absence. Our results indicate that mutations in *dnaC* restore recombination, DNA repair, and normal levels of uninduced *lexA*-repressed gene expression to a *priA2::kan* strain. This suggests that an altered DnaC may initiate

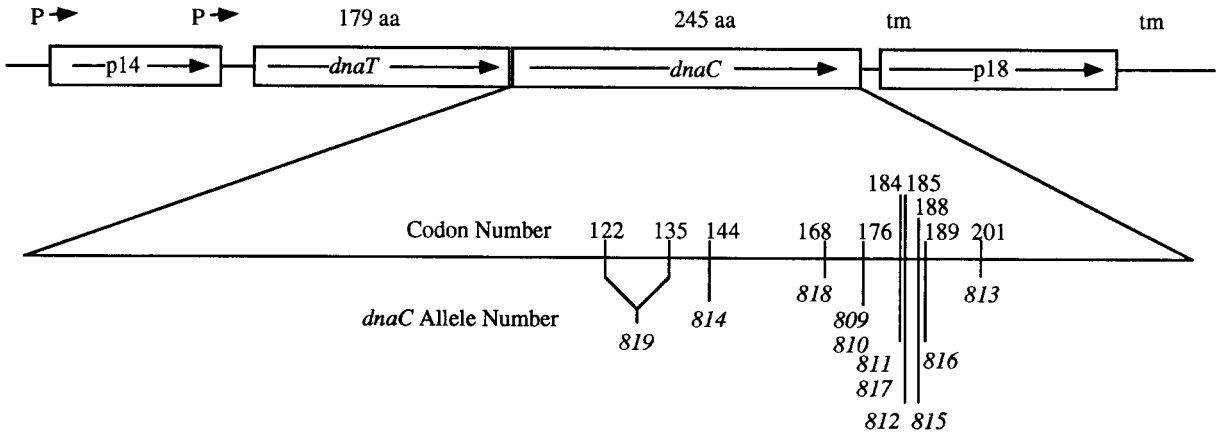

FIGURE 4.—The location of the *priA2::kan* suppressor mutations in *dnaC*. The figure shows a drawing of the *dnaTC* operon and surrounding region. Rectangles indicate open reading frames, and arrows indicate direction of transcription with the arrowhead at the 3′ end. Promoters (P with an arrow) and terminators of transcription (tm) have been mapped (MASAI and ARAI 1988). Open reading frames of expressed genes with no known function are called p14 and p18. The number of amino acids in *dnaT* and *dnaC* are indicated above the genes. The position in number of codons from the start of the *dnaC* of the suppressing mutation(s) is shown with the allele number of each mutant. Allele numbers are shown for those mutations with different DNA sequence changes. The actual change is reported in Table 3.

primosome assembly in recombination and DNA repair (see below). The mutations in *dnaC* that suppress the *priA2::kan* UV^S phenotype all map in the carboxy terminal half of the protein between codons 121 and 202 (Figure 4). These changes are expected to add a PriA compensatory activity without inactivating vital functions. This hypothesis is consistent with our inference that *dnaC809* and the other suppressor mutations are dominant to $dnaC^+$.

What clues to *priA*'s function can be garnered from the suppression of *priA2::kan* by *dnaC809*? DnaC loads the DnaB helicase onto ssDNA either at *oriC* in a DnaA, HU-dependent fashion (BRAMHILL and KORNBERG 1988) or at regions of ssDNA that have *pas* sites in a PriA, PriB, PriC, DnaT-dependent manner (ALLEN and KORNBERG 1993). DnaB, DnaC, and ATP form a complex *in vitro* (WAHLE *et al.* 1989a). It is from this complex that DnaC is thought to deliver DnaB to the DNA. DnaC is able to inhibit the ATPase activity of DnaB (WAHLE *et al.* 1989a,b; ALLEN and KORNBERG 1991). Higher than normal amounts of DnaC can either suppress certain mutant alleles of *dnaB* (SCLAFANI and WECHSLER 1981) or slow the rate of DNA synthesis at existing DNA replication forks in wild-type cells [presumably through an interaction with DnaB (SKARSTAD and WOLD 1995)]. Thus DnaC protein seems to recognize specific protein-DNA structures where DnaB should be loaded, to load DnaB onto the DNA, and to modulate DnaB's activity both before and after loading onto the DNA. Since in particular cases, PriA is important for the loading of DnaB in a DnaC-dependent fashion away from *oriC*, we suggest that DnaC loads DnaB at PriA-nucleic acid structures that are necessary for recombination and DNA repair. In a *priA2::kan* strain this loading does not occur. Hence the cells are UV^S and Rec^-. DnaC809 corrects this defect allowing the loading of DnaB at these structures in a PriA-independent manner.

Where does PriA function? *In vitro,* PriA requires a *pas* site to load on the ssDNA coated with SSB (SHLOMAI and KORNBERG 1980). This is based on the fact that PriA was purified by its ability to interact with the *pas* site on the ϕX174 chromosome. No *pas* sites however have yet been found on the bacterial chromosome (MASAI *et al.* 1994). If, however, *pas* is a structure that ssDNA phages and plasmids have adopted to recruit PriA and related proteins to their lifestyles, then the physiologically relevant structure in *E. coli* that PriA may recognize and bind may be different. We suggest that PriA may recognize a D-loop, a Holliday junction or a combination of the two mediated by Holliday structure-binding proteins such as RuvA (WEST and CONNOLLY 1992). These structures might also contain SSB. This suggestion gives PriA an important physiological role in recombinational DNA repair and cell viability as dictated by its genetic phenotypes. It also provides a way in which PriA could act in a *pas*-sequence independent fashion.

How can the activity of PriA, DnaB and DnaC function in recombination and DNA repair? Recombination and DNA replication are coupled during late or recombination-dependent replication of T4. In this process, *uvsX* and *gp32* products are used to make D-loops, which in turn are used to initiate replication forks (reviewed in KREUZER and MORRICAL 1994). Hence, there is precedent for recombination intermediates being used to initiate DNA replication that consists of both leading and lagging strand synthesis.

We suggest that PriA, DnaB and DnaC load the DNA replication machinery to initiate semi-conservative DNA replication at or near a protein-DNA structure involved in recombination. A model of recombination that re-

quires semi-conservative DNA replication is "ends out" RecBCD-mediated recombination (SMITH 1991). Here, the ends of the exogenote are tailored by RecBCD to allow RecA to utilize them for D-loop formation. Just downstream of these D-loops, a replication fork is postulated to be assembled that then allows for semi-conservative DNA replication away from the ends of the exogenote. A specific mechanism for how PriA may function in the loading of the replication machinery to make a replication fork in inducible stable DNA replication has been proposed (ASAI *et al.* 1994; MASAI *et al.* 1994). This mechanism is akin to the mechanism for the PriA initiation of semi-conservative DNA replication at the *ColE1* origin (KORNBERG and BAKER 1992). In all these examples, DNA replication occurs postsynaptically.

We would also like to point out that PriA's role in recombination may be just the loading of DnaB, or just primosome assembly, and may not require the creation of a replication fork at an appropriate protein-nucleic acid structure as discussed above.

We thank TOKIO KOGOMA, KEN MARIANS and PEARL NURSE for strains and plasmids used in this work. We also thank LYNN ZECHIEDRICH and MURTY MADIRAJU for critically reading the manuscript. This research was supported by grant AI-05371 from the National Institutes of Health.

LITERATURE CITED

ALLEN, G. C., and A. KORNBERG, 1991 Fine balance in the regulation of DnaB helicase by DnaC protein in replication in *Escherichia coli*. J. Biol. Chem. **266:** 22096–22101.

ALLEN, G. C., and A. KORNBERG, 1993 Assembly of the primosome of DNA replication in *Escherichia coli*. J. Biol. Chem. **268:** 19204–19209.

ASAI, T., and T. KOGOMA, 1994 D-loop and R-loop: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. J. Bacteriol. **176:** 1807–1812.

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.

BRAMHILL, D., and A. KORNBERG, 1988 Duplex opening by *dnaA* protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell **52:** 743–755.

KORNBERG, A., and T. BAKER, 1992 *DNA Replication*. W. H. Freeman and Company, New York.

KREUZER, K. N., and S. W. MORRICAL, 1994 Initiation of DNA replication, pp. 28–42 in *Molecular Biology of Bacteriophage T4*, edited by J. KARAM. American Society for Microbiology, Washington, DC.

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.

LEE, E. H., H. MASAI, G. C. ALLEN and A. KORNBERG, 1990 The *priA* gene encoding the primosomal replicative n' protein of *Escherchia coli*. Proc. Natl. Acad. Sci. USA **87:** 4620–4624.

LITTLE, J. W., S. H. EDMISTON, L. Z. PACELLI and D. W. MOUNT, 1980 Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA **77:** 3225–3229.

LLOYD, R. G., and G. J. SHARPLES, 1992 Genetic analysis of recombination in prokaryotes. Curr. Opin. Genet. Dev. **2:** 683–690.

MASAI, H., and K.-I. 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.

MASAI, H., T. ASAI, Y. KUBUTA, K.-I. ARAI and T. KOGOMA, 1994 *Escherichia coli* PriA protein is essential for inducible and constitutive stable DNA replication. EMBO J. **13:** 5338–5345.

MOUNT, D. W., K. B. LOW and S. J. EDMISTON, 1972 Dominant mutations (*lex*) in *Escherchia coli* K-12 which affects radiation sensitivity and frequency of ultaviolet light-induced mutations. J. Bacteriol. **112:** 886–893.

NURSE, P., R. J. DIGATE, K. H. ZAVITZ and K. J. MARIANS, 1990 Molecular cloning and DNA sequence analysis of *Escherichia coli priA*, the gene encoding the primosomal protein replication factor Y. Proc. Natl. Acad.Sci. USA **87:** 4615–4619.

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.

SANDLER, S. J., and A. J. CLARK, 1993 Use of high and low level overexpression plasmids to test mutant alleles of the *recF* gene of *E. coli* K-12 for partial activity. Genetics **135:** 643–654.

SCLAFANI, R. A., and J. A. WECHSLER, 1981 Deoxyribonucleic acid initiation mutation *dnaB252* is suppressed by elevated *dnaC*$^+$ gene dosage. J. Bacteriol. **146:** 418–421.

SHLOMAI, J., and A. KORNBERG, 1980 A prepriming DNA replication enzyme of *Escherichia coli* II. Actions of protein n': a sequence specific, DNA-dependent ATPase. J. Biol. Chem. **255:** 6794–6798.

SHYAMALA, V., and G. F.-L. AMES, 1989 Amplication of bacterial genomic DNA by the polymerase chain reaction and direct sequencing after asymmetric amplification: application to the study of periplasmic permeases. J. Bacteriol. **171:** 1602–1608.

SINGER, M., T. A. BAKER, G. SCHNITZLER, S. M. DEISCHEL, M. GOEL *et al.*, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:** 1–24.

SKARSTAD, K., and S. WOLD, 1995 The speed of the *Escherichia coli* fork *in vivo* depends on the DnaB:DnaC ratio. Mol. Microbiol. **17:** 825–831.

SMITH, G. R., 1991 Conjugational recombination in E. coli: myths and mechanisms. Cell **64:** 19–27.

TANAKA, K., T. ROGI, H. HIASA, D.-M. MIAO, Y. HONDA *et al.*, 1994 Comparative analysis of functional and structural features in the primase-dependent priming signals, G sites, from phages and plasmids. J. Bacteriol. **176:** 3606–3613.

TWIGG, A. J., and D. SHERRATT, 1980 Trans-complementable copy-number mutants of plasmid ColE1. Nature **283:** 216–218.

WAHLE, E., R. S. LASKEN and A. KORNBERG, 1989a The *dnaB-dnaC* replication protein complex of *Escherichia coli* I. Formation and properties. J. Biol. Chem. **264:** 2463–2468.

WAHLE, E., R. S. LASKEN and A. KORNBERG, 1989b The *dnaB-dnaC* replication protein complex of *Escherichia coli* II. Roles of the complex in mobilizing *dnaB* dunctions. J. Biol. Chem. **264:** 2469–2475.

WEST, S. C., and B. CONNOLLY, 1992 Biological roles of the *Escherichia coli* RuvA, RuvB, and RuvC proteins revealed. Mol. Microbiol. **6:** 2755–2759.

WICKNER, S., and J. HURWITZ, 1974 Conversion of ϕX174 viral DNA to double-stranded form by purified *Escherichia coli* protein. Proc. Natl. Acad. Sci. USA **71:** 4120–4124.

WILLETTS, N. S., A. J. CLARK and B. LOW, 1969 Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97:** 244–249.

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.

ZIEG, J., and S. R. KUSHNER, 1977 Analysis of genetic recombination between two partially deleted lactose operons of *Escherichia coli* K-12. J. Bacteriol. **131:** 123–132.

Communicating editor: G. R. SMITH