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PriA Is Essential for Viability of the *Escherichia coli* Topoisomerase IV *parE10*(Ts) Mutant

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The *parE10*(Ts) mutation, which renders *Escherichia coli* thermosensitive for growth by inactivation of the essential *E. coli* topoisomerase topo IV, is lethal at all temperatures when PriA, the main replication restart protein, is absent. This lethality is suppressed by the activation of a PriA-independent replication restart pathway (*dnaC809* mutation). This result suggests that topo IV acts prior to full-chromosome replication completion.

Dissociation of the replisome upon replication arrest creates a need for replication reinitiation at a nonorigin sequence. In bacteria, the key protein of the main replication restart pathway is PriA, which targets primosome assembly to replication forks and to recombination intermediates (13, 15). Although *priA* single mutants are viable, mutant cells in which the frequency of replication arrest is increased require PriA for viability, presumably because of a need for efficient replication restart. For example, PriA is essential for viability in cells mutated for replication proteins such as the holoenzyme polymerase III subunit HolD (4), the replicative helicase Rep (14, 17), or the gyrase subunit GyrB (5). Conversely, a requirement for *priA* for viability is likely to indicate an increased need for primosome assembly and therefore an increased frequency of replication arrest and replisome dissociation.

The *Escherichia coli* type II topoisomerase topo IV is composed of two subunits encoded by the *parC* and *parE* genes (7). Its main role is the decatenation of fully replicated chromosomes in the terminus region (reviewed in reference 10). It also contributes to the steady-state level of negative supercoiling (20). In addition, topo IV can act at replication forks; simultaneous inactivation of gyrase and topo IV leads to immediate replication arrest, indicating that when the gyrase activity is compromised, topo IV is essential for replication elongation (8). Whether topo IV also acts during replication progression when gyrase is fully active has remained an open question. We report here the observation that partial inactivation of topo IV renders PriA essential for viability; the need for replication restart when topo IV activity is compromised indicates that topo IV acts prior to replication completion even in the presence of gyrase.

Since *priA* mutants acquire at a high rate the compensatory mutations that suppress the growth defect, strains were constructed in the presence of a plasmid, pAM-*priA*, which carries the wild-type *priA* gene and replicates from a conditional origin (5). Replication of pAM-*priA* is under the control of the *lac* operator and thus requires the presence of the inducer isopropyl-β-D-thiogalactopyranoside (IPTG). Propagation of pAM-*priA*-containing cells in the absence of IPTG prevents plasmid replication and allows the isolation of plasmidless clones (5). The *parE10*(Ts) mutation (7) was cotransduced from the original W3110 genetic background into JJC40 (wild type) and the isogenic mutant JJC1398 [*sfiA11 priA2*::*kan*(pAM-*priA*)] (5). Three different markers adjacent to *parE10*(Ts) were used: *tolC210*::Tn*10*, *metC162*::Tn*10*, and *zgj3075*::Tn*10* (12). The cotransduction linkages between the *parE10*(Ts) mutation and the Tet^r (Tn*10*) markers were identical in JJC40 and JJC1398, indicating that the pAM-*priA* plasmid does not affect the viability of *parE10*(Ts) mutants (data not shown). The *parE10*(Ts) mutation prevented the growth of JJC40 and JJC1398 at 37 and 42°C, whereas it did not affect colony formation at 30°C.

For curing cells of the plasmid, overnight cultures grown at 30°C in Luria broth (LB) containing 500 μg of IPTG/ml and 60 μg of spectinomycin/ml were diluted 1,000-fold in minimal medium (M63; 0.2% glucose, 0.2% Casamino Acids). After 8 h of growth at 30°C, appropriate dilutions were plated on minimal medium plates devoid of IPTG to count *priA2*::*kan* cells and on spectinomycin-IPTG-containing plates to count plasmid-containing cells. The plates were incubated for 4 days at 30°C. *sfiA11 priA2*::*kan*(pAM-*priA*) cultures propagated for 8 h in the absence of IPTG contained (i) 0.5×10^7 to 2×10^7 cells per ml that were able to form colonies on spectinomycin-IPTG-containing plates, which harbored pAM-*priA*, and (ii) 0.5×10^8 to 2×10^8 cells per ml that were able to form colonies on minimal medium plates devoid of IPTG (Table 1). Less than 1% of the latter colonies could grow on minimal medium containing spectinomycin and IPTG, indicating that they had lost the plasmid during propagation in liquid medium or early during colony formation. Analysis of such clones by PCR and phenotype tests showed that they were indeed *priA* mutants (5). When the same experiment was performed with *parE10*(Ts) *priA2*::*kan*(pAM-*priA*) cells, 0.5×10^7 to 2×10^7 cells per ml were able to form colonies on spectinomycin-IPTG-containing plates, and hence they still harbored pAM-*priA*. In contrast, 10^4 to 10^5 microcolonies were obtained on minimal medium devoid of IPTG; they did not regrow on any

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TABLE 1. *parE10*(Ts) *priA* and *parE10*(Ts) *priA recA* strains are nonviable, whereas the *parE10*(Ts) *priA dnaC809* strain is viable

Strain	Relevant genotype	No. of viable cells on IPTG-free medium/ml[a]
JJC1398	*priA2*::*kan*	0.5×10^8 to 2×10^8
JJC1897	*priA2*::*kan parE10*(Ts) *tolC*::Tn*10*	<10
JJC2269	*priA2*::*kan parE10*(Ts) *metC*::Tn*10*	<10
JJC2270	*priA2*::*kan parE10*(Ts) *zgj*::Tn*10*	<10
JJC1898	*priA2*::*kan parE10*(Ts) *tolC*::Tn*10 dnaC809*	1×10^8 to 5×10^8
JJC2272	*priA2*::*kan parE10*(Ts) *metC*::Tn*10 dnaC809*	1×10^8 to 5×10^8
JJC2271	*priA2*::*kan parE10*(Ts) *zgj*::Tn*10 dnaC809*	1×10^8 to 5×10^8
JJC2092	*priA2*::*kan parE10*(Ts) *tolC*::Tn*10*Δ*recA*	<10

[a] Segregation experiments were performed at 30°C as described in the text.

medium (Table 1). In some experiments, rare colonies of normal size were obtained on minimal medium; however, those were still resistant to spectinomycin (Spe^r; the antibiotic resistance phenotype encoded by pAM-*priA*) and PriA$^+$ and were likely to result from the integration of pAM-*priA* into the chromosome. Similarly, no *parE10*(Ts) *priA2*::*kan* mutant could be isolated when *parE10*(Ts) *priA2*::kan(pAM-priA) cells were grown at 30°C in LB and plated on LB plates, indicating that the *parE10*(Ts) *priA* double mutant is also lethal in rich medium (LB). These experiments indicate that the *parE10*(Ts) *priA2*::*kan* mutant is nonviable at 30°C.

The growth defect of *priA* mutants is suppressed by mutations in the *dnaC* gene, such as *dnaC809*, that allow DnaC to load the DnaB replicative helicase in the absence of PriA (16, 18). The *dnaC809* mutation was used to test whether the lethality of the *priA parE10*(Ts) double mutant is suppressed by the activation of this PriA-independent pathway of primosome assembly. The *parE10*(Ts) mutation was introduced by cotransduction with adjacent Tn*10* markers into a *sfiA priA2*::*kan dnaC809*(pAM-*priA*) strain (JJC1767, isogenic to JJC40 and JJC1398; the *dnaC809* allele was cotransduced with Thr$^+$ and identified by *Hin*fI restriction digestion of a *dnaC* PCR fragment; Table 1). The resulting *priA2*::*kan dnaC809 parE10*(Ts) (pAM-*priA*) mutants were grown in minimal medium at 30°C in the absence of IPTG to prevent pAM-*priA* replication. A total of 1×10^8 to 5×10^8 plasmidless *priA2*::*kan dnaC809 parE10*(Ts) clones per ml were obtained after 8 h of growth (Table 1). The *parE10*(Ts) *priA2*::*kan dnaC809* plasmidless colonies were similarly obtained on rich (LB) and minimal media at 30°C. As expected from the thermosensitive phenotype conferred by the *parE10*(Ts) mutation, no colonies were obtained upon plating at 37 or 42°C. The observation that the *dnaC809* mutation suppresses the requirement for PriA in the *parE10*(Ts) mutants at 30°C indicates a requirement for efficient primosome assembly in this topo IV mutant, via either the PriA pathway or the DnaC809 pathway.

PriA is essential for RecBCD-RecA-catalyzed homologous recombination (16). The requirement for PriA may therefore result from a requirement for homologous recombination in *parE10*(Ts) mutants. *recA*, *recB*, and *ruvABC* mutations were used to test this hypothesis. These mutations inactivate different steps of homologous recombination: RecBC is required for the formation of single-stranded DNA at double-strand ends and for RecA loading on DNA, RecA catalyzes homology search and strand exchange, and RuvABC resolves the four-way junctions formed by strand exchange (reviewed in reference 9). Δ*recA*::Kan^r (5) and Δ*recBCD*::Kan^r (11) derivatives of the *parE10*(Ts) *tolC*::Tn*10* mutant and a Δ*ruvABC*::Cm^r derivative (17) of the *parE10*(Ts) *zgj*::Tn*10* mutant were constructed by P1 transduction. Transductants were obtained at 30°C with the expected efficiency; they were sensitive to UV irradiation and thermosensitive for growth as expected (data not shown). Inactivation of homologous recombination by *recBC* or a *ruvABC* mutation did not compromise the growth of the *parE10*(Ts) mutant at 30°C (Table 2). The *parE10*(Ts) *recA* mutant exhibited a 30-fold-reduced plating efficiency that was not observed with *recB* and *ruvABC* mutations and may therefore result from a need for SOS induction in the *parE10*(Ts) mutant. The viability of *parE10*(Ts) recombination mutants indicates that the lethality of the *priA parE10*(Ts) double mutant at 30°C does not result from a need for RecBCD-RecA-catalyzed homologous recombination. To independently confirm this result, a Δ*recA938*::Cm^r mutation was introduced in the *parE10*(Ts) *priA2*::*kan*(pAM-*priA*) mutant, and the resulting *parE10*(Ts) *priA2*::*kan* Δ*recA938*::Cm^r(pAM-*priA*) mutant was propagated in the absence of IPTG to cure the plasmid. No plasmidless cells were recovered, indicating that PriA is required in the *parE10*(Ts) mutant even in a *recA* background, in which homologous recombination is prevented (Table 1). This experiment confirms that PriA is not required in the *parE10*(Ts) mutant to restart from a recombination intermediate. We propose that the primosome is efficiently assembled directly at blocked forks in the *parE10*(Ts) mutant.

The colethality of *parE10*(Ts) and *priA* mutations is reminiscent of the colethality of *gyrB*(Ts) and *priA* (5). The requirement for PriA in *gyrB* mutants is similarly suppressed by *dnaC809* and is not accompanied by a requirement for homologous recombination proteins. During replication progression, the increased linking number caused by the unwinding of parental DNA by replicative helicases can take the form of positive supercoils downstream of replication forks, which accumulate in the absence of gyrase and can be converted into positive precatenates that link the two daughter chromatids. It was proposed that *gyrB* mutations and *priA* inactivation are colethal because of the accumulation of positive supercoils that block replication progression (5). Dissociation of the replication machinery allows the conversion of positive supercoils into precatenates and replication restarts in a PriA-dependent way. Topo IV is more efficient in vitro in the decatenation of full catenates or semicatenates than in the relaxation of positively supercoiled DNA and is essential in vivo for the decatenation of fully replicated molecules (6, 19). However, single-molecule experiments indicated that topo IV is indeed able to efficiently

TABLE 2. *parE10*(Ts) recombination mutants are viable[a]

Strain	Relevant genotype	CFU at OD_{650} of ~0.5
JJC1896	*parE10*(Ts)	$1.5 \times 10^8 \pm 2.8 \times 10^7$
JJC2090	*parE10*(Ts) *recA*	$3.9 \times 10^6 \pm 3.3 \times 10^6$
JJC2091	*parE10*(Ts) *recB*	$4.8 \times 10^7 \pm 9.4 \times 10^6$
JJC2276	*parE10*(Ts) *ruvABC*	$1.1 \times 10^8 \pm 2.1 \times 10^7$

[a] Cells were grown in LB to an optical density at 650 nm of ~0.5. Appropriate dilutions were plated on LB plates and incubated for 48 h at 30°C. The threefold decrease in plating efficiency due to the *recB* mutation was also observed in ParE$^+$ cells (data not shown).

remove positive supercoils (3). We propose that fork progression is arrested in the *parE10*(Ts) strain at 30°C by the accumulation of positive supercoils downstream of the progressing fork, which renders PriA essential for growth.

If both topo IV and gyrase can remove positive supercoils, inactivation of only one of them should be compensated by the action of the other one. In contrast, a decreased activity of either GyrB or ParE creates a need for PriA (5; this work). This paradox can be resolved if topo IV and gyrase do not have exactly the same target. For example, they may not act in the same chromosome domain, with topo IV removing positive supercoils specifically in the terminus region of the chromosome and gyrase removing them in the origin and intermediate domains. Indeed, the terminus of the chromosome differs from the origin and the intermediate domains by several features. Among those is a lack of the repeated element BIME 2, a sequence recognized by gyrase (1), and a reduced frequency of gyrase activity (2). The low activity of gyrase in the terminus region of the chromosome may be compensated by the presence of active topo IV.

In conclusion, the observation that replication restart is essential in the *parE10*(Ts) mutant indicates that topo IV plays an essential role during the replication fork's progression prior to chromosome replication completion; further work is needed to clearly identify this role.

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