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::Tn10, metC162::Tn10, and zgi3075::Tn10 (12). The cotransduction linkages between the parE10(Ts) mutation and the Tet' (Tnl0) 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 × 10⁷ to 2 × 10⁷ cells per ml that were able to form colonies on spectinomycin-IPTG-containing plates, which harbored pAM-priA, and (ii) 0.5 × 10⁸ to 2 × 10⁸ 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)s priA2::kan(pAM-priA) cells, 0.5 × 10⁷ to 2 × 10⁷ cells per ml were able to form colonies on spectinomycin-IPTG-containing plates, and hence they still harbored pAM-priA. In contrast, 10⁴ to 10⁷ microcolonies were obtained on minimal medium devoid of IPTG; they did not regrow on any
mutants were grown in minimal medium at 30°C. A total of 1.1 × 108 plasmidless priA2::kan 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) priA::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::kan mutant could be isolated when parE10(Ts) priA::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) priA::kan mutant is nonviable at 30°C.

The lethality of priA mutants in parE10(Ts) double mutants is suppressed by the activation of this PriA-independent pathway of primosome assembly. The parE10(Ts) mutation was introduced by cotransduction with adjacent Tn10 markers into a sfiA priA::kan dnaC809(pAM-priA) strain (JJC1767, isogenic to JJC40 and JJC1398; the dnaC809 allele was cotransduced with Thr° and identified by Hinfl 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 × 109 to 5 × 109 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). ΔrecA::Kan° (5) and ΔrecBCD::Kan° (11) derivatives of the parE10(Ts) tolC::Tn10 mutant and a ΔruvABC::Cm° derivative (17) of the parE10(Ts) zyg::Tn10 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, a ΔrecA938::Cm° mutation was introduced in the parE10(Ts) priA2::kan(pAM-priA) mutant, and the resulting parE10(Ts) priA2::kan ΔrecA938::Cm°(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 co lethality of parE10(Ts) and priA mutations is reminiscent of the co lethality 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 co lethal 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

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<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>CFU at OD600 of ~0.5</th>
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<tbody>
<tr>
<td>JJC1896</td>
<td>parE10(Ts)</td>
<td>1.5 × 10^8 ± 2.8 × 10^7</td>
</tr>
<tr>
<td>JJC2090</td>
<td>parE10(Ts) recA</td>
<td>3.9 × 10^4 ± 3.3 × 10^4</td>
</tr>
<tr>
<td>JJC2091</td>
<td>parE10(Ts) recB</td>
<td>4.8 × 10^7 ± 9.4 × 10^7</td>
</tr>
<tr>
<td>JJC2276</td>
<td>parE10(Ts) ruvABC</td>
<td>1.1 × 10^8 ± 2.1 × 10^7</td>
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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 sequence recognized by gyrase (1), and a reduced frequency of positive supercoils specifically 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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REFERENCES