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Coupling between DNA Replication and Cell Division Mediated by the FtsA Protein in *Escherichia coli*: a Pathway Independent of the SOS Response, the "TER" Pathway

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Inhibition of DNA synthesis prevented the recovery of cell division in filaments of D-3R [*ftsA3*(Ts) *recA56*] returned to the permissive temperature. The FtsA protein may be a signal involved in the "TER" pathway, a series of events that coordinate cell division with DNA replication, that is independent of the SOS pathway.

Under physiological conditions, replication and division are tightly coordinated in *Escherichia coli*, with anucleate cells being very rarely formed (8). Inhibition of DNA synthesis blocks cell division but not cell growth (2, 7). The existence of mechanisms that coordinate bacterial DNA replication and cell division has been suggested (7). A termination protein, required for septation and synthesized at the end of the replication period, has been postulated as a signal to coordinate these two processes (15). Proof of the existence of such a protein has been difficult to obtain, because the treatments used to inhibit DNA synthesis (17, 25) induce the SOS response (10, 16, 27). One of the SOS genes is *sulA*, whose product has been suggested to interact with the *sulB-ftsZ* (17) gene product (12, 14). Inactivation of FtsZ, a protein required in the initial stages of septation (19, 20), results in a block of cell division (14).

Inhibition of DNA synthesis in *recA* strains results in filamentation and the appearance of anucleate cells. These cells form a part of the population which is smaller than that expected if the SOS pathway were the only mechanism coordinating replication and division (8, 9, 13). It has been described recently (1, 11) how treatments that block the termination of DNA replication inhibit the division of cells not undergoing their D period (time from the end of DNA replication to cell separation into two daughter cells).

Tormo et al. (24, 25) have shown that FtsA, a structural component of the septum (26) having a size of 48 kilodaltons (18) and that is synthetized and required during the late stages of the cell cycle (6), needs DNA replication for its synthesis. They suggest that FtsA acts in the coupling between DNA replication and cell division (24, 25). Nevertheless, induction of the SOS response was not excluded in their experiments, thereby casting doubts on their conclusions.

To obviate this problem, we have constructed an *ftsA3*(Ts) *recA56* strain. The *recA56* allele was introduced into strain D-3 [*ftsA3*(Ts)] (25) by conjugation with JC10240 (HfrP045 *recA56 srlC*300::Tn*10 thr300 ilv318 rpsE300*) (21) as previously described (4). The exconjugants were selected for tetracycline resistance and checked for UV sensitivity. The *ftsA3*(Ts) *recA56* strain, D-3R, ceased dividing at 42°C with no residual divisions upon transfer to the restrictive temperature, as expected from an *ftsA*(Ts) strain (data not shown), and enabled us to study the relationships between replication and septation mediated by FtsA in the absence of the SOS response.

The recovery of cell division in temperature-induced filaments of *ftsA*(Ts) strains, i.e., D-2 [*ftsA2*(Ts)], D-3 [*ftsA3*(Ts)], and D-3R [*ftsA3*(Ts) *recA56*], was measured (Fig. 1) both in the presence and absence of replication, under conditions in which the SOS response could be induced (*recA*$^+$ strains) or not (*recA56*). Nalidixic acid was used to inhibit DNA replication at a final concentration of 80 μg/ml. For the induction of the SOS response, cultures were irradiated at 10 J/m^2 after filtration and suspension in M9 minimal medium (3). Irradiated cells were then diluted in an equal volume of doubly concentrated nutrient broth plus thymine (100 μg/ml) and kept in the darkness to prevent photoreactivation. Particle number and optical density were measured as previously described (25).

After a 60-min incubation at 42°C, cells of D-2, D-3, and D-3R increased their mass 4.4-fold. Under these conditions, they fulfilled all of the requirements for cell division but one, i.e., the synthesis or action of the FtsA protein. As the *ftsA2* phenotype in D-2 is thermoreversible (i.e., the FtsA protein inactivated at 42°C regains its activity upon transfer to 30°C), division of temperature-induced filaments could occur at the permissive temperature in the absence of protein synthesis (26) and even in the absence of chromosome replication (Fig. 1) (24).

Under the same experimental conditions, D-3, an *ftsA3(Ts)* thermoirreversible strain, was unable to divide after DNA synthesis inhibition (25) (Fig. 1) and exhibited a transient cell division inhibition after UV irradiation (Fig. 1). These results suggested that active DNA replication was required for FtsA synthesis, but an SOS effect could not be excluded.

To avoid effects on cell division mediated by the induction of the SOS response, the action of nalidixic acid on temperature-induced filaments of strain D-3R [*ftsA3*(Ts) *recA56*] was studied. Filaments of strain D-3R, induced at 42°C and returned to the permissive temperature, were able to divide without undergoing transient cell division inhibition after UV irradiation, as expected from their *recA56* background (Fig. 1). They eventually ceased dividing, however, as expected from their lack of a repair system. Nevertheless,

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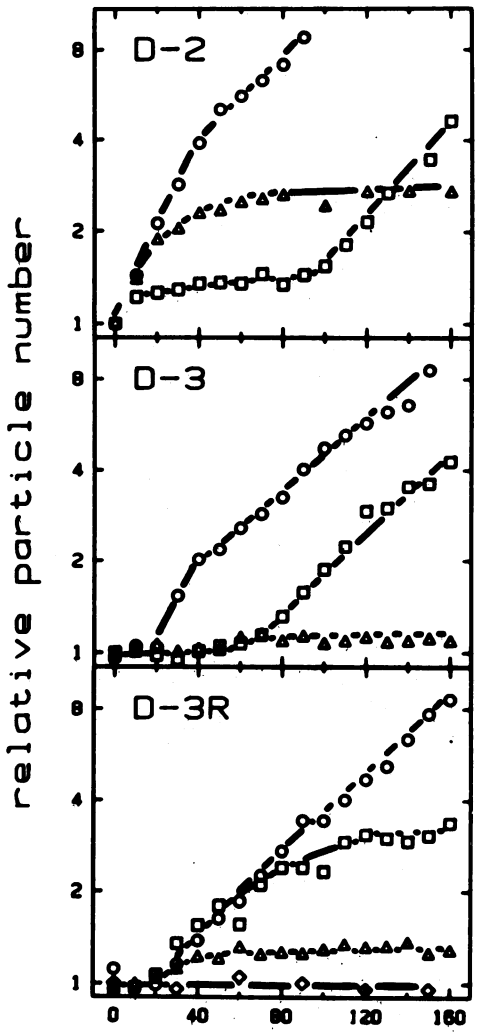

FIG. 1. Recovery of cell division in temperature-induced filaments of strains D-2, D-3, and D-3R. Exponentially growing cultures at 30°C were incubated for 60 min at 42°C. After this period, the cultures were split into several portions that were transferred to 30°C (time zero). One was UV irradiated (10 J/m^2) (□), nalidixic acid was added to a second portion (△), another portion was subjected to both UV irradiation (10 J/m^2) and nalidixic acid (◇), and the remaining portion was not treated (○). Particle number was monitored throughout the experiment. Initial particle numbers were as follows: 4.1×10^7 (D-2), 1.5×10^7 (D-3), and 1.1×10^7 (D-3R).

division of these filaments was markedly inhibited if nalidixic acid was added when they were returned to 30°C. Thus, the inhibition of cell division caused by nalidixic acid in D-3 and D-3R filaments cannot be ascribed to the induction of the SOS response. The most plausible explanation for this behavior is that FtsA required replication for its synthesis but not for its action. A few residual divisions occurred at 30°C in filaments of strain D-3R, even in the presence of nalidixic acid. Nevertheless, recovery of cell division was completely prevented when both UV irradiation and nalidixic acid treatments were used (Fig. 1).

If chromosome replication were required for FtsA synthesis (Fig. 1), it would be expected that a pulse of chromosome replication would allow some FtsA protein to be synthesized and, therefore, some divisions to occur. After transfer to the permissive temperature, cell division of D-3 and D-3R filaments occurred in increasing amounts when DNA synthesis was allowed to proceed at various intervals within the 20 min required for cell division recovery. The results (Fig. 2) indicated that temperature-induced filaments of strains D-3 and D-3R could only divide after a pulse of DNA replication at the permissive temperature. The presence of the *recA56* mutation in strain D-3R ensured that replication was a genuine requirement for division. The smaller amounts of division found when nalidixic acid was present were not a consequence of the SOS-dependent division inhibition.

Nalidixic acid interferes with DNA gyrase, thereby af-

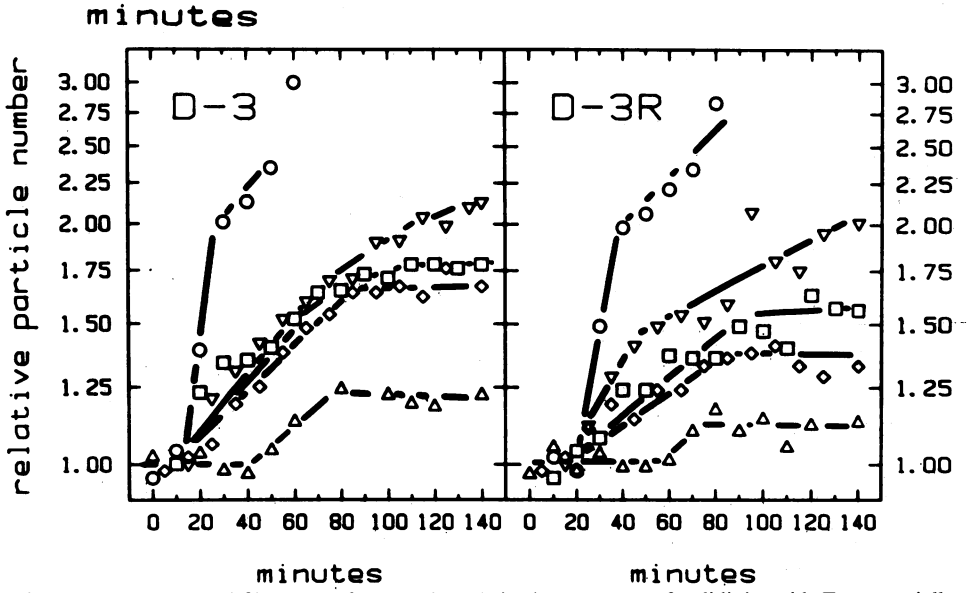

FIG. 2. Division of temperature-induced filaments of D-3 and D-3R in the presence of nalidixic acid. Exponentially growing cultures at 30°C were incubatd for 60 min at 42°C. At the end of this period (time zero), the cultures were split into several portions that were transferred to 30°C. Nalidixic acid was added at 0 min (△), 5 min (◇), 10 min (□), and 15 min (▽) or not added (○). Initial particle numbers were as follows: 3.2×10^7 (D-3), and 1.1×10^7 (D-3R).

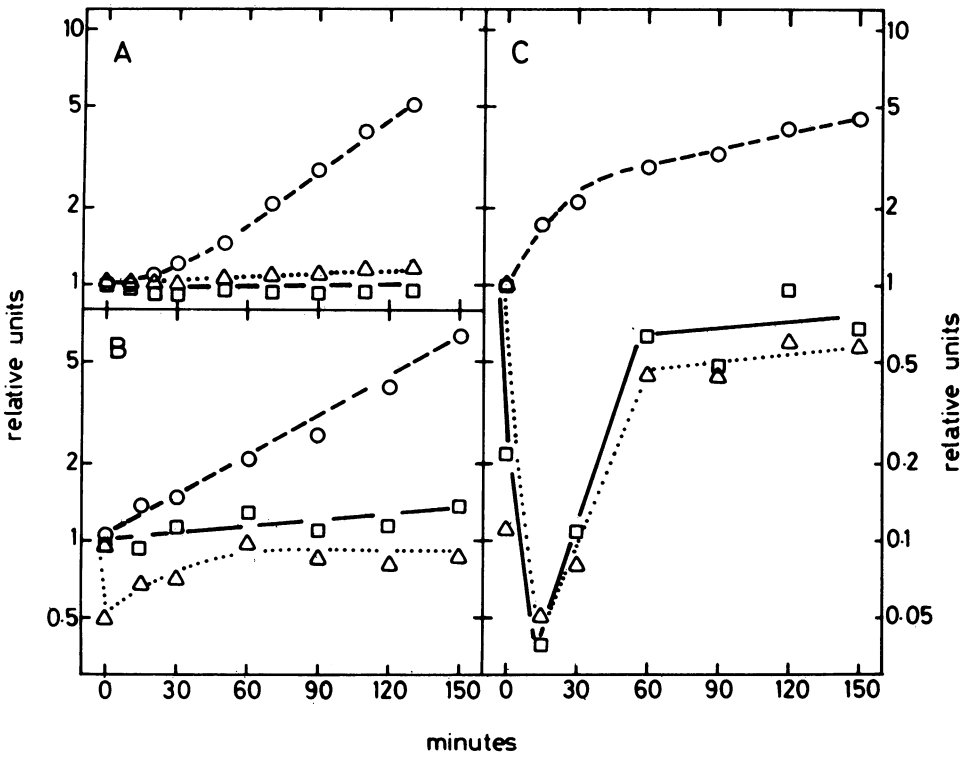

FIG. 3. Patterns of DNA and RNA increase in strain D-3R after treatment with nalidixic acid and hydroxyurea. Cultures growing exponentially at 30°C were incubated for 60 min at 42°C. After this period, the cultures were split into three portions that were transferred back to 30°C (time zero). Nalidixic acid (80 μg/ml) was added to one (△), hydroxyurea (15 mg/ml) was added to the second (□), and the third portion was not treated (○). One-milliliter samples were withdrawn at the times indicated, and synthesis of DNA and RNA was measured by pulse-labeling for 5 min at 30°C with either 5 μCi of [^{3}H]thymidine (47 Ci/mmol) or 5 μCi of [^{3}H]uridine (29 Ci/mmol), respectively. (A) Particle numbers during the experiment; (B) counts incorporated into RNA; (C) counts incorporated into DNA. Initial values, panel A, 1.3 × 10^7 particles per ml; panel B, 1.0 × 10^4 cpm; panel C, 6.2 × 10^3 cpm.

fecting the transcription of certain genes (22, 23). It is also known that the expression of the *sulB* gene may be affected by the supercoiling state of the DNA (R. Gayda and A. Markovitz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H128, p. 129).

To determine whether the state of supercoiling affected the expression of the *ftsA* gene, we studied the recovery of cell division in D-3R filaments after treatment with hydroxyurea, another inhibitor of DNA synthesis that prevents the formation of DNA precursors but is not directly related to the gyrase activity.

Both nalidixic acid and hydroxyurea drastically inhibited DNA replication during the first 20 min after the return to the permissive temperature (the time required for the expression of the *ftsA* gene) (Fig. 3). The effect of both treatments on RNA synthesis differed, however. Nalidixic acid inhibited RNA synthesis (50% of the control during the first 20 min at 30°C), but RNA inhibition was not found in the hydroxyurea treatment. Furthermore, the level of RNA synthesis remained at a constant level as expected from the inhibition of DNA replication. Nevertheless, inhibition of DNA replication by hydroxyurea caused a total block in the recovery of cell division in D3-R filaments.

From our results in Fig. 2 and 3, a short pulse of DNA synthesis appeared, then, to be a requirement for *ftsA3* filaments to resume division. Considering our present results in light of previous data on FtsA, all of the evidence is consistent for FtsA, a septal protein, having a role in a termination protein-like signal or perhaps being a sort of termination protein itself. We therefore propose the term "TER" pathway to designate these controls exerted by chromosomal replication on proteins required for the completion of cell division.

How DNA replication induces the synthesis of FtsA remains an interesting question. As *ftsA* maps far away from the terminus of replication, no structural activation of its expression could be invoked. Perhaps its dependence on replication for expression could be mediated by other products coded by genes located near the terminus or could be a consequence of the activation of *oriX* (5).

It is worth noting that the products of two genes that map in the 2.5-min division cluster and that are involved in the development of the septum are sensitive, at the level of expression (*ftsA*) or activity (*ftsZ*), to DNA synthesis or damage.

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