The *ftsA* Gene Product: a Possible Connection between DNA Replication and Septation in *Escherichia coli*

By ANTONIO TORMO,* CONCEPCIÓN FERNÁNDEZ-CABRERA AND MIGUEL VICENTE

Instituto de Biología Celular, Consejo Superior de Investigaciones Científicas, Velázquez 144, 28006 Madrid, Spain

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The study of *Escherichia coli* strain D-2, which harbours the *ftsA2(ts)* allele, has shown that temperature-induced filaments of this strain can divide, at 30 °C, in the absence of DNA replication and translation. Strain D-2 is thermosensitive during a period coincident with that in which the termination protein should be synthesized and exert its action. The *ftsA* gene product, which participates in the structure of the septum, needs for its synthesis a short period of DNA replication. The FtsA protein could be involved in a mechanism that coordinates chromosome replication and cell division by a pathway different from and independent of the SOS-induced response.

INTRODUCTION

Division in *Escherichia coli* is a discontinuous event but, during balanced growth, it is well coordinated with several growth processes such as duplication of the length, mass, volume and DNA content of the cell. These processes as such are largely continuous during the lifespan of a cell, except for initiation and termination of chromosome replication. The dependence of cell division on DNA replication has been described (Clark, 1968; Helmstetter & Pierucci, 1968). Inhibition of the rounds of chromosome replication is followed by a stop in cell division. Jones & Donachie (1973) postulated the existence of effector(s) required for cell division that would be synthesized at the end of a round of replication, their synthesis, in turn, requiring active replication and transcription. This effector(s) was named termination protein(s) and is supposed to coordinate chromosome replication and cell division under normal growth conditions.

Mutants affected in this postulated termination protein have not been described up to now and, also, a molecular species having the properties of a termination protein has not been found (Lutkenhaus *et al.*, 1979). The results of Donachie *et al.* (1979) and Tormo *et al.* (1980) have shown that the period of action and synthesis of the *ftsA* gene product occurs during the late stages of the *E. coli* cell cycle. Moreover, the synthesis and/or action of the FtsA protein needs a short pulse of DNA synthesis. In *ftsA* mutants a thermal shock during the period of synthesis and action of the termination protein prevents cell division even if all other requirements for division are fulfilled (Donachie *et al.*, 1979; Tormo *et al.*, 1980). These results suggest that the role of FtsA is in some respects similar to that of a termination protein (Tormo *et al.*, 1980). Other results (Tormo & Vicente, 1984; A. Tormo, M. Vicente & M. A. de Pedro, unpublished results) have shown that FtsA is located in the inner membrane, having a structural role in the formation of the division septa.

In this work the physiology of *E. coli* strains D-2 and D-3 containing different *ftsA* alleles has been studied. The results obtained support those previously described for *ftsA* mutants (Donachie *et al.*, 1979; Tormo *et al.*, 1980) and suggest that the role of FtsA is similar to that of a termination protein. Our results corroborate the need for DNA synthesis for the recovery of septation in filaments of *ftsA3*, but they show that this is a requirement for the synthesis of the *ftsA* gene product and not for its action.

METHODS

Bacterial strains. The *E. coli* K12 strains used in this work, D-2 and D-3, are derivatives of OV-2 (Donachie *et al.*, 1976): *F⁻ leu ilv his thyA(deo) ara(Am) lacI25(Am) galE trp(Am) tsx(Am) tyrT(supF A81ts)*. D-2: as OV-2, *leu⁺ ftsA2(ts)* (Tormo & Vicente, 1984). D-3: as OV-2, *leu⁺ ftsA3(ts)* (Tormo *et al.*, 1980).

Media and growth conditions. Cells were grown in LB medium supplemented with 20 µg thymine ml⁻¹. Minimal medium was M9 (Clowes & Hayes, 1968) with glucose as a carbon source (4 mg ml⁻¹); the auxotrophic requirements of the strain, adenine, uracil and cytosine (20 µg ml⁻¹ each) and Casamino acids (5 mg ml⁻¹) were also added.

Cells were grown at the permissive temperature (30 °C) in liquid media in a shaking water-bath so that balanced growth was maintained during several doublings (no less than four) before the beginning of the experiment. Procedures for cell parameter measurements, photomicrography of nucleoids and chromosome alignment have been described previously (Tormo *et al.*, 1980).

Inhibitors. DNA replication was inhibited by nalidixic acid (80 µg ml⁻¹, final concn) or by hydroxyurea (15 mg ml⁻¹, final concn). Transcription was inhibited with rifampicin (500 µg ml⁻¹, final concn). Protein synthesis was inhibited with chloramphenicol (200 µg ml⁻¹, final concn).

RESULTS AND DISCUSSION

Physiological characterization of strain D-2 (ftsA2)

Strain D-2 contains a temperature-sensitive division mutation, shown by complementation analysis to be an allele of *ftsA* and termed *ftsA2(ts)* (Tormo & Vicente, 1984). The kinetics of increase in OD₄₅₀, particle number and viable counts at 30 °C occurred at a rate similar to that found with the parent strain OV-2 (Fig. 1). At 42 °C (restrictive temperature) OD₄₅₀ increased at a higher rate (as expected in a temperature shift-up) but particle number and viable counts remained constant, showing that no cell division occurred (Fig. 1). DNA synthesis and nucleoid segregation suffered no detectable alterations at 42 °C as filaments of strain D-2 had several nucleoids uniformly distributed throughout their length (results not shown). The fact that no cell division occurred upon transfer to the restrictive temperature corroborated the need for an active FtsA protein until cell separation, as has already been suggested (Tormo *et al.*, 1980).

As expected from the behaviour of other *ftsA* alleles (Donachie *et al.*, 1979; Tormo *et al.*, 1980) it was found that strain D-2 is thermosensitive during the last stages of the division cycle and that cells in which all the requirements for division are present, except expression of the *ftsA* gene, fail to divide under restrictive conditions (results not shown). The behaviour of strain D-2 is therefore compatible with that of a strain with a thermosensitive termination protein.

Recovery of division at 30 °C in temperature-induced filaments of strain D-3 (ftsA3): requirement for replication and transcription

Filaments of strain D-3 (*ftsA3*), a temperature-irreversible *ftsA* mutant, cannot divide upon return to 30 °C if replication is inhibited by nalidixic acid or if translation is blocked by chloramphenicol when the culture is returned to permissive conditions (Tormo *et al.*, 1980). But if a short pulse of replication and translation is allowed after the return of strain D-3 filaments to 30 °C, they are then able to divide (Tormo *et al.*, 1980). The fact that translation is needed for the recovery of division in strain D-3 is easily explained if the inactivation of the *ftsA3* gene product were irreversible. The need for DNA replication is harder to understand. Among various possibilities three seem plausible to explain the observed behaviour. First, the inhibitor, nalidixic acid, could inhibit both replication and cell division. Second, inhibition of DNA replication by nalidixic acid treatment can induce the SOS response (Little & Mount, 1982; Witkin, 1976), the inhibition of cell division being one of its side effects. Third, the *ftsA* gene product may need for its synthesis and/or action a short period of replication. An experiment was designed to test the first possibility. Temperature-induced filaments of strain D-3 were transferred to permissive conditions and replication was inhibited at the moment of transfer, or 20 min later, by hydroxyurea (an inhibitor of replication not related in its molecular structure or action to nalidixic acid). The results (Fig. 2) showed that cell division was inhibited if replication was blocked by hydroxyurea at the time of transfer but not if it was blocked 20 min later. Therefore, we can reasonably rule out the first possibility, a side effect of nalidixic acid on cell

Fig. 1

Fig. 1. Kinetics of growth of strain D-2 at 30 and 42 °C. A culture growing at 30 °C in LB medium supplemented with thymine was divided in two. One half was kept at 30 °C (open symbols) and the other was shifted to 42 °C at time 0 (filled symbols). Increases in OD₄₅₀ (Δ, ▲), cell number (○, ●) and viable counts (□, ■) were measured at the times indicated. Initial values were: 2.6×10^7 particles ml⁻¹, 1.5×10^7 viable cells ml⁻¹ and 0.1 OD₄₅₀ units.

Fig. 2

Fig. 2. Recovery of division in temperature-induced filaments of strain D-3. A culture growing exponentially at 30 °C in LB medium supplemented with thymine was shifted to 42 °C. After 30 min at this temperature, the culture was divided into several parts that were shifted back to 30 °C at time 0. Hydroxyurea was added at 0 min (□) or at 20 min (■); rifampicin was added at 0 min (○), at 10 min (●) or at 20 min (▲); one part was transferred to 30 °C without any inhibitor as a control (Δ). The initial value was 3.5×10^7 particles ml⁻¹.

division, as it seems unlikely that hydroxyurea would cause the same side effects as nalidixic acid. That transcription is required to allow cell division upon return to permissive conditions is in accordance with our interpretation that the *ftsA3* gene product is irreversibly inactivated at 42 °C (Tormo & Vicente, 1984), as was also found when rifampicin was used to inhibit transcription. Filaments of strain D-3 therefore needed translation and transcription to resume division.

Almost any inhibition of DNA replication induces the SOS response (Little & Mount, 1982; Witkin, 1976), one effect of which is a transient inhibition of cell division. Although nalidixic acid has been reported to induce the SOS response (Little & Mount, 1982), it is not yet known whether every block in DNA replication inhibits cell division as a consequence of the SOS response or through some other mechanism that could coordinate replication and cell division. The SOS response induced by different treatments has different pleiotropic effects (Barbe *et al.*, 1983). Moreover, thymine starvation, reported to induce the SOS response (Little & Mount, 1982), does not inhibit cell division immediately, permitting a high residual division. It was, therefore, an open question whether the absence of division of temperature-induced filaments of strain D-3 at 30 °C when DNA synthesis was inhibited (Tormo *et al.*, 1980 and Fig. 2) was due to a possible SOS response or whether replication was needed for the synthesis and/or action of FtsA. The first explanation did not seem very plausible because filaments of strain D-3 could not divide if replication was inhibited at the time the cells were transferred to the permissive temperature but they readily divided if DNA synthesis was inhibited a few minutes later (Tormo *et al.*, 1980 and Fig. 2). It is difficult to understand why the SOS response, if it was induced by nalidixic acid, could inhibit cell division when it was added at the time of transfer of the filaments to permissive conditions but not when added 5 min later. The possibility that FtsA needs a short pulse of replication for its synthesis and/or action seems, from these results, more plausible.

Fig. 3

Fig. 3. Recovery of division in temperature-induced filaments of strain D-2[*ftsA2(ts)*, thermoreversible]. A culture growing at 30 °C in LB medium supplemented with thymine was shifted to 42 °C. After 30 min at this temperature the culture was divided into several parts that were shifted back to 30 °C at time 0. Hydroxyurea (●), nalidixic acid (■) or chloramphenicol (□) were added at time 0 to different parts; one part without inhibitor was the control (○); a fifth part was kept at 42 °C (▲). The initial value was 2.3×10^7 particles ml^{-1} .

Fig. 4

Fig. 4. Particle number increase in temperature-induced filaments of strain D-2 in the absence of replication. A culture of D-2 growing in LB medium supplemented with thymine was divided into several parts that were shifted to 42 °C and kept at this temperature for 5, 30 or 60 min. After these periods of preincubation at the restrictive temperature the different portions were divided in two and returned to 30 °C in the presence or absence of nalidixic acid ($80 \mu\text{g ml}^{-1}$ final concentration). The kinetics of increase in cell number was followed. The residual increase in cell number in the presence of nalidixic acid (●) and the increase in cell number after 25 min at 30 °C in the absence of the inhibitor (○) were plotted versus the time of preincubation at 42 °C. The initial value was 2.5×10^7 particles ml^{-1} .

Recovery of division at 30 °C in temperature-induced filaments of strain D-2 is independent of translation and replication

Strain D-2 contains an *ftsA2* allele in which the gene product has been described as reversibly thermosensitive (Tormo & Vicente, 1984). Temperature-induced filaments of this strain are therefore able to divide upon return to permissive conditions, even in the absence of protein synthesis. To find out whether the first division that occurs upon transfer of these filaments to permissive conditions was dependent on replication, the kinetics of cell division in temperature-induced filaments of strain D-2 in the presence of different inhibitors was measured. The results (Fig. 3) indicate that division of strain D-2 filaments, under permissive conditions, could occur not only in the absence of translation, as already described (Tormo & Vicente, 1984), but also in the absence of replication (i.e. in the presence of nalidixic acid or hydroxyurea). As the *ftsA2* gene product is thermoreversible the rate of cell division of filaments returned to 30 °C should increase as a function of the time spent at 42 °C. The residual division of these filaments in the absence of replication should also increase as a function of the time spent at 42 °C. The results in Fig. 4 show that this was the case.

These results do not support the possibility that the SOS-associated inhibition of division is responsible for the lack of residual division in the presence of nalidixic acid in filaments when returned to 30 °C. We would have to admit in such a case that the *ftsA2* allele renders the cells resistant to SOS-mediated cell division inhibition, and therefore that septa on which the *ftsA* gene product has interacted (Tormo & Vicente, 1984) are resistant to the action of SulA, the SOS division inhibitor (Gottesman *et al.*, 1981; Huisman & D'Ari, 1981; Mizusawa & Gottesman,

1983), or that *ftsA* is a target of the SOS division inhibition pathway as is *sulB* (Lutkenhaus, 1983). Neither case would explain why strain D-3 filaments divide if replication is not inhibited immediately after the return to permissive conditions.

It has been suggested that inhibition of chromosome replication without alterations of other structural or physiological functions of the chromosome should not inhibit cell division (Helmstetter *et al.*, 1979). Inhibition of DNA synthesis results in the induction of the SOS response (Little & Mount, 1982), one of its effects being the inhibition of cell division. From the results obtained by several authors it seems that a pathway other than SOS, and independent of it, should coordinate cell division and replication. It has been reported (Inouye, 1971) that in the absence of replication (thymidine starvation or nalidixic acid treatment) *recA* strains divide and produce anucleated cells. The SOS response is inhibited in these strains as the RecA protein does not acquire the specific SOS protease function upon DNA damage or synthesis inhibition. After UV irradiation *recA* strains, as expected, do not undergo transient filamentation (Burton & Holland, 1983). After chromosome replication inhibition, *recA* cells divide once, or at most twice, producing DNA-less cells and nucleated filaments. If the SOS pathway were the only control between replication and division, there should be continuous division of nucleated cells and no filamentation. It seems then that an additional mechanism to inhibit cell division after chromosome replication has to exist. Similar results are obtained if, instead of *recA*, *lexA* strains are used (Howe & Mount, 1975, 1978). Since in these strains the *lexA* gene product is not cleaved by the RecA protease, the SOS response is blocked. Inhibition of DNA synthesis in *lexA* strains leads to the formation of filaments, 85% of these being able to divide only once and 15% twice or more. Again, the existence of a SOS independent pathway coordinating replication and division arises.

A plausible explanation for the results obtained with strains D-3 and D-2 is that replication is a requirement for FtsA synthesis but not for its action. If the FtsA action requires replication, cell division in the absence of replication should be prevented in strain D-2 filaments. Conversely, if the action of FtsA does not require replication, a residual division of strain D-2 filaments at 30 °C in the absence of DNA synthesis should be observed. As this latter alternative is found experimentally (Figs 3 and 4) it seems that the synthesis, but not the action, of the *ftsA* gene product requires replication.

The existence of a protein that may affect cell division but is not dependent on the SOS response has been detected in *lon* but not in *lon*⁺ strains (Schoemaker *et al.*, 1982). This finding accords with the existence of a division pathway, involving *ftsA*, that is not directly regulated by the SOS inducible genes, in contrast to the situation for *sulB* (equivalent to *ftsZ*), the neighbour gene of *ftsA* (Lutkenhaus, 1983). We have shown that the *ftsA* gene product participates in the formation of the septum (Tormo & Vicente, 1984); it would not be surprising to find that septation proteins are responsible for some of the regulatory functions needed by the cell to coordinate replication and cell division, so as to grow and divide in a balanced fashion. The relationship between the SOS-mediated inhibition of division and the action of *ftsA* remains to be elucidated.

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