Induction of Cell Division in a Temperature-sensitive Division Mutant of *Escherichia coli* by Inhibition of Protein Synthesis

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SUMMARY

Synchronous cells of the thermosensitive division-defective *Escherichia coli* strain MACI (*divA*) divided at the restrictive temperature (42 °C) if they were allowed to grow at 42 °C for a certain period before protein synthesis was inhibited by adding chloramphenicol (CAP) or rifampicin. The completion of chromosome replication was not required for such *divA*-independent division. Synchronous cells of strain MACI divided in the presence of an inhibitor of DNA synthesis, nalidixic acid, if they were shifted to 42 °C and CAP or rifampicin was added after some time; cells of the parent strain MC6 (*divA*⁺) treated in the same way did not divide. These data suggest that coupling of cell division to DNA synthesis depends on the *divA* function. The ability to divide at 42 °C, whether or not chromosome termination was allowed, was directly proportional to the mean cell volume of cultures at the time of CAP addition, suggesting that cells have to be a certain size to divide under these conditions. The period of growth required for CAP-induced division had to be at the restrictive temperature; when cells were grown at 30 °C, in the presence of nalidixic acid to prevent normal division, they did not divide on subsequent transfer to 42 °C followed, after a period, by protein synthesis inhibition. A model is proposed in which the role of *divA* as a septation initiator gene is to differentiate surface growth sites by converting a primary unregulated structure, with the capacity to make both peripheral wall and septum, to a secondary structure committed to septum formation.

INTRODUCTION

Cell growth and cell division are coordinated processes which are linked in *Escherichia coli* by chromosome replication. Initiation of chromosome replication occurs when the ratio of cell mass (or volume) to the number of chromosome origins reaches a value which is constant over a wide range of growth conditions (Donachie, 1968; Pritchard, 1968). Cell division follows after an interval which encompasses the time (C) for replication and the time (D) between the end of replication and physical cell separation (Cooper & Helmstetter, 1968). The values of C and D are constant at different growth rates (1 to 3 generations h⁻¹; Cooper & Helmstetter, 1968; Helmstetter & Pierucci, 1976). In *E. coli* K12, C is also constant in slow-growing cells (less than 1 generation h⁻¹; Chandler, Bird & Caro, 1975). Under normal conditions, completion of chromosome replication is a prerequisite for division (Clark, 1968; Helmstetter & Pierucci, 1968). The coupling between DNA replication and cell division is still poorly understood and the nature of the timing control systems for the division processes that take place during the D period is unknown (Donachie, Jones & Teather, 1973). Another unexplained observation is that, for doubling times between 27 and 45 min, a fixed period of protein synthesis which normally occurs concurrent with

chromosome replication but can be uncoupled from it, is required for division (Pierucci & Helmstetter, 1969). Previous work (Pedro, Llamas & Cánovas, 1975) on a thermosensitive division-defective strain of *E. coli*, MAC1, showed that the gene (*divA*) mutated in this organism appears to control cell division during a period which lasts 40 min and ends 20 min before division, a period concurrent with DNA replication, and that its function can be uncoupled from replication. The same work also supports the view that *divA* is involved in the initiation of the division processes which usually take place during the D period. Finally, it has been proposed that *divA* has a role in fixing the time for the protein synthesis period necessary for division in *E. coli*. This paper shows that when the *divA* function is impaired, cell division can be carried out under certain conditions. The completion of a round of DNA replication is not a prerequisite for this *divA*-independent cell division.

METHODS

The isolation and properties of the *Escherichia coli* mutant MAC1, a thermosensitive, division-defective, *str-r* derivative of *E. coli* K12 F⁻ *thyA dra drm thi thr leu pro* (MC6), have been described previously (Pedro *et al.*, 1975). Synchronous cultures growing at 30 °C on LB medium (generation time τ approx. 50 min) were used. The materials and methods used to obtain these cultures by the membrane elution technique and the methods employed for cell number determinations and for temperature shifts from 30 to 42 °C, and vice versa, have also been described (Pedro *et al.*, 1975). To calculate the mean cell volume of cultures, the cell volume distribution was determined by serial counting of the particles between two size thresholds, using a model ZBI Coulter counter. Each lower threshold was set at the same size as the preceding upper threshold.

RESULTS

Division of strain MAC1 cells at 42 °C in the presence of protein synthesis inhibitors

The experiments described in this paper arose from an unexpected observation illustrated in Fig. 1(a). To test whether the product of the gene mutated in strain MAC1 could revert to the active form after heat treatment, a synchronous culture was given a long heat shock at 42 °C, starting when the cells were close to becoming heat resistant (20 min after cell elution). The culture was then incubated at 30 °C and chloramphenicol (CAP) was added. Protein synthesis stopped in about 2 min. Cell division occurred under these conditions but it had different characteristics from the control culture to which no CAP had been added (Fig. 1). Division began after 20 min with CAP compared with 50 min in the control, and the number of particles in the presence of CAP more than doubled, whereas only 60 to 70% of the control cells were able to divide as observed previously (Pedro *et al.*, 1975). These results suggested that the protein synthesis-independent division of strain MAC1 might not depend on the *divA* product. This appears to be true because cells divided in the presence of CAP even at 42 °C (Fig. 1 b). Division at the restrictive temperature required both CAP and an incubation period at 42 °C before antibiotic addition; cells without CAP or cells to which CAP was added after only 10 min at 42 °C did not divide. When the experiments illustrated in Fig. 1 were repeated using rifampicin (400 µg ml⁻¹) instead of CAP, similar results were obtained.

The ability to divide at 42 °C in the presence of CAP was directly proportional to the length of the previous incubation period without the protein synthesis inhibitor (Fig. 2a). A certain period of incubation after cell elution was necessary before the CAP was added

Fig. 1. Division of strain MACI at 30 °C and 42 °C in the presence of CAP. (a) A synchronous culture growing at 30 °C on LB medium was subjected to 60 min at 42 °C starting 20 min after cell elution. The culture was then returned to 30 °C and divided into two parts: CAP ($200 \mu\text{g ml}^{-1}$) was immediately added to one part (○) but not to the other (●). Initial particle counts ml^{-1} were 5×10^5 .

(b) A synchronous culture growing at 30 °C on LB medium was shifted to 42 °C 20 min after cell elution; the culture was divided into two parts 60 min later and CAP ($200 \mu\text{g ml}^{-1}$) was added to one part (○) but not to the other (●). An identical culture was also shifted to 42 °C 20 min after cell elution and CAP ($200 \mu\text{g ml}^{-1}$) was added 10 min later (△). Initial particle counts ml^{-1} were 5×10^5 (○, ●) and 4×10^5 (△).

Fig. 2. Acquisition of ability to divide at 42 °C in the presence of CAP in strain MACI. A number of synchronous cultures growing at 30 °C on LB medium were shifted to 42 °C at the time of cell elution (●) or 20 min after (○). CAP ($200 \mu\text{g ml}^{-1}$) was added to these cultures at different times and the maximum number of particles in each culture was measured and plotted against (a) the time of CAP addition or (b) the mean cell volume of cultures at CAP addition. To calculate relative values, those corresponding to the mean cell volume and particle counts ml^{-1} at the time of cell elution were taken as 1.0.

to detect CAP-induced divisions; this was about 20 min in synchronous cultures shifted to 42 °C at elution and approximately 35 min in cultures shifted to 42 °C 20 min after elution (Fig. 2a). The ability to divide at 42 °C was also directly proportional to the mean cell volume measured in the cultures at the time of CAP addition (Fig. 2b). A critical volume, which was almost the same in cultures shifted to 42 °C at elution or 20 min later, was required before divisions were detected. Thus, cell growth to a critical size is necessary for CAP-induced division at 42 °C.

Fig. 3. Lack of inhibition by NA of strain MAC1 division at 42 °C. NA ($80 \mu\text{g ml}^{-1}$) was added at the time of cell elution to a synchronous culture growing at 30 °C on LB medium and 20 min later the temperature was shifted to 42 °C; the culture was divided into two parts after another 60 min and CAP ($200 \mu\text{g ml}^{-1}$) was added to one part (○) but not to the other (●). Initial particle counts ml^{-1} were 6×10^8 .

Fig. 4. Acquisition of ability to divide at 42 °C in the presence of NA in strain MAC1. NA ($80 \mu\text{g ml}^{-1}$) was added at the time of cell elution to a number of synchronous cultures growing at 30 °C on LB medium and 20 min later the temperature was shifted to 42 °C. CAP ($200 \mu\text{g ml}^{-1}$) was added to these cultures at different times and the maximum number of particles in each culture was measured and plotted against (a) the mean cell volume of cultures at CAP addition or (b) the time of CAP addition. Relative values were calculated as in Fig. 2.

Division of strain MAC1 cells at 42 °C in the presence of nalidixic acid

Completion of chromosome replication is not a prerequisite for *divA*-independent cell division. This was shown in synchronous cultures of strain MAC1 to which nalidixic acid (NA) was added at the time of cell elution. Cells did not divide when they were kept at 30 °C. However, if the temperature was shifted to 42 °C and CAP (or rifampicin) was added after some time, the number of cells increased (Fig. 3). No division was observed without the protein synthesis inhibitor. Cell growth at 42 °C in the presence of NA was variable; the mean cell volume under these conditions after a fixed incubation period was different in different cultures. Nevertheless, the ability to divide at 42 °C in the presence of NA was proportional to the mean cell volume at the time of CAP addition (Fig. 4a), but not to the length of the incubation period before adding the protein synthesis inhibitor (Fig. 4b). Division without completion of chromosome replication was only possible when the *divA* product was inactivated; cell division in the presence of NA was not observed in synchronous cultures of strain MAC1 growing at 30 °C or of the parent strain MC6 growing at 42 °C, even

Fig. 5. Lack of CAP-induced division of strain MACI at 42 °C caused by prolonged previous incubation at 30 °C. A synchronous culture growing on LB medium at 30 °C, to which NA (80 $\mu\text{g ml}^{-1}$) had been added at the time of cell elution, was divided into two parts 20 min after elution. One part was immediately shifted to 42 °C (●) and the other was shifted 40 min later (○). CAP (200 $\mu\text{g ml}^{-1}$) was added to both parts 60 min after their corresponding temperature change. Initial particle counts ml^{-1} were 2.8×10^5 .

if CAP (or rifampicin) was added when the mean cell volume was rather large (data not shown).

Previous studies on strain MACI behaviour (Pedro *et al.*, 1975) suggested that division is controlled by the *divA* gene for a period (PA) of about 40 min starting 55 to 60 min before physical cell separation. If this is true, PA for the first division round ends 30 min after the elution of synchronous cultures (τ approx. 50 min) and PA for the second division round must start 40 min after elution. In the experiments reported above, the temperature shift-up was probably carried out before both the end of the first PA and the beginning of the second PA. The experiment illustrated in Fig. 5 was designed to test if strain MACI could divide in the presence of NA when the temperature shift-up was carried out after the theoretical initiation of PA for the second division round. A synchronous culture to which NA was added at elution, was shifted to 42 °C about one generation time later and CAP was added after another 60 min. No division was observed in this culture in contrast to a parallel control culture shifted to 42 °C 20 min after elution (Fig. 5).

DISCUSSION

Apparently, the control of septation by the *divA* gene can be by-passed in strain MACI cells if they are allowed to reach a critical size at the restrictive temperature before protein synthesis is inhibited. The *divA*-independent division process cannot be blocked by inhibition of DNA synthesis which suggests that coupling of cell division to chromosome replication depends on the *divA* function.

Induction of *E. coli* cell division by protein synthesis inhibition (or by inhibition of peptide bond formation) has previously been reported by Zusman, Inouye & Pardee (1972) in strain MX74T2 *ts52*, a thermosensitive division-defective mutant. As in strain MACI, there is a cumulative effect of the incubation time at the restrictive temperature on the level of subsequent CAP-induced division. A striking difference between the strains is that strain *ts52* appears to maintain the normal coupling of cell division to DNA replication. The strain *ts52* and strain MACI mutations map at about 32 and 3 min respectively on the *E. coli* linkage

Fig. 6. Diagram to illustrate the hypothetical changes affecting surface growth sites and a possible pattern of cell division in a cell of strain MAC1 grown on LB medium at 30 °C (τ approx. 50 min) and incubated under the same conditions or at the restrictive temperature (42 °C). Symbols represent different states of the surface growth sites. ●, Sites not yet affected by the *divA* product; these sites have a primary unregulated structure with a capacity to make both peripheral wall and septum, the latter ability being elicited by protein synthesis inhibition. □, Sites partially modified by the *divA* product in a process which starts 60 min before division and ends 20 min before division; these sites are strictly engaged in cell elongation and cannot be induced to form a septum by protein synthesis inhibition. ∇, Sites after completion of the *divA* controlled process; these sites are committed to septum formation but are dependent on chromosome termination.

map. It can be inferred (Zusman *et al.*, 1972) that control by the gene mutated in strain *ts52* ends very early in the cell cycle since cells from an exponential culture growing with a generation time of 30 min stopped dividing in about 55 min when shifted to 42 °C. This feature is also different in strain MAC1; *divA* control ends 20 min before division (Pedro *et al.*, 1975).

Induction of cell division by protein synthesis inhibition cannot be unequivocally interpreted with the available information. On the basis of previous results (Pedro *et al.*, 1975) and present observations, it is conceivable that the *divA* product function is to differentiate surface growth zones (potential division sites) by transforming a primary unregulated structure, with the capacity to make both peripheral wall and septum, to a secondary structure committed to septum formation. The possible sequence of changes affecting surface growth zones is illustrated in Fig. 6. It is assumed that septum formation by the surface growth zones with the primary unregulated structure can be elicited by protein synthesis inhibition, i.e. when cells are not forced to extend in length due to the absence of mass increase. This assumption is based on Pritchard's model of cell division (Pritchard, 1974) in which division occurs when synthetic capacity for wall formation exceeds that required to accommodate the increment in mass. The formation of new surface growth sites maintaining the primary structure should be necessary to induce strain MAC1 division by CAP or rifampicin addition. Such sites must be created by allowing cell growth to a certain size at 42 °C, whether DNA replication is terminated or not. This model predicts that after incubation of strain MAC1 at 42 °C, cells with a volume less than that critical for the formation of new surface growth sites cannot be induced to divide by CAP addition, whereas those which exceed that volume must divide and produce up to three cells if we assume that

Fig. 7. Comparison of the division of strain MACI at 42 °C in the presence of CAP with the expected cell division assuming that cells which were able to divide under these conditions made two septa. Data were from the experiment reported in Fig. 2, in which synchronous cultures were shifted to 42 °C at the time of cell elution. Theoretical values (○) were calculated on the basis of cell volume distribution at the time of CAP addition; cells with a volume of less than 2 units were assumed not to divide, whereas cells of more than 2 volume units were assumed to produce up to 3 new cells. Experimental values (●) are those reported with the same symbols in Fig. 2(a). One volume unit is equal to the mean cell volume of the culture at the time of elution.

two new surface growth sites should be formed (Fig. 6). This prediction can be tentatively tested with the available data because cell growth of a synchronous culture of strain MACI at 42 °C was variable, producing cells of different sizes (data not shown). Experimental values for CAP-induced division after various periods at 42 °C are compared with the expected cell division according to the proposed hypothesis in Fig. 7. Both theoretical and experimental values are similar, though not equal. However, CAP partially inhibits the protein synthesis-independent division of strain MACI cells which are in the D period (Pedro *et al.*, 1975). The data in Fig. 1(a) are also consistent with the suggested hypothesis that cells induced to divide by protein synthesis inhibition yield more than two daughter cells; CAP induced division after a heat shock at 42 °C more than doubled the number of particles although only 60 to 70 % of the cells were able to divide in the absence of CAP. The results shown in Fig. 5 also agree with the model. When the *divA* product was inactivated one generation time after cell elution, the new potential division sites should have already lost the primary unregulated structure and should not be induced to septate by CAP addition.

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