Cell Division of a Double Mutant of *Escherichia coli* Bearing Lesions of *recA* and Thermosensitive Division Deficiency

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In some bacteria including *Escherichia coli*,¹⁾ *Salmonella typhimurium*²⁾ and *Caulobacter crescentus*³⁾ cell division has been shown to couple with chromosome replication. When DNA synthesis is disturbed by the addition of inhibitors or by deprivation of thymine from the medium of a thymine-requiring strain, cells stop dividing and form filaments.

In 1971, Inouye⁴⁾ reported that *recA* strain of *E. coli* still continue to divide even in the absence of DNA synthesis. Therefore, cell division in this mutant is uncoupled with chromosome replication. However, it is unknown whether this division, unlinked to DNA synthesis, is regulated or accomplished through a process similar to normal division.

On the other hand, there are several thermosensitive mutants of *E. coli* deficient in cell division.⁵⁾ One of them, ts 20,⁶⁾ grows normally at 30°C and stops dividing immediately after a temperature shift to 42°C. Macromolecular syntheses including DNA, RNA, and protein are grossly normal at restrictive temperature.

Therefore, a double mutant bearing both lesions of thermosensitivity and *recA* was prepared, and the process of division was examined.

The division mutant ts20 (F⁻ *thr leu trp his thi thy ara lac gal xyl mtl str*^r) derived from *E. coli* K12 JE 1011 was mated with *E. coli* K12 KL 16-99 (Hfr *rel-1 thi recA*), and Thy⁻ His⁺ colonies were isolated. From the recombinants, a strain tsR20 which is thermosensitive, labile to mitomycin C and deficient in recombination ability was selected and used for further study.

Cells were cultured in an enriched M9 medium containing 5.8 g of Na₂HPO₄, 3.0 g of KH₂PO₄, 0.5 g of NaCl, 1.0 g of NH₄Cl, 11 mg of CaCl₂, 246 mg of MgSO₄·7H₂O, 4 g of glucose, 2 g of casamino acids, 20 mg of tryptophan, 2 mg of thiamine and 10 mg of thymine per liter of distilled water. The cell number were assayed by a Coulter counter model Zb after the appropriate dilution of the culture with filtered saline.

As reported previously,⁶⁾ cells of ts 20 proliferated exponentially at 30°C, and stopped dividing after a temperature shift to 42°C. When the culture was

shifted back from 42° to 30°C, the cell number increased more rapidly than the normal culture at 30°C, indicating accumulation of division potential during incubation at restrictive temperature. No increase was observed when chloramphenicol (CP) was added at the time of the temperature was shift back. Similar results were obtained with tsR20.

When thymine was deprived from the medium, no increase in cell number was observed with ts20 even at the permissive temperature. However, a *recA* derivative of ts20, tsR20 continued to divide in the absence of thymine, and the cell number increased linearly for three hours after the deprivation. This process of division, unlinked to DNA synthesis, was shown to be thermosensitive as well as the normal division. The cells formed filaments as long as those observed in the presence of thymine during incubation at 42°C, as indicated by morphological examination and by increased absorbance.

The number of tsR20 cells without thymine increased when the culture was shifted back from 42° to 30°C, and this division was also sensitive to CP. However, in contrast to the case in the presence of thymine, no burst in cell division was observed. Therefore, no accumulation of division potential was shown during incubation

FIG. 1. Effect of Temperature and Thymine on Division of ts20 (A) and tsR20 (B).

An exponentially growing culture in enriched M9 at 30°C was filtered and washed. Cells were resuspended in M9 medium supplemented with (open symbols) or without (closed symbols) thymine. Two parts of the culture were incubated at 30°C (○ and ●) and the other two parts were shifted to 42°C (Δ and ▲). After 120 min of incubation at 42°C, fractions of the latter were shifted back to 30°C with (▽ and ▼) or without (□ and ■) 100 μg/ml of CP. The mark (×) represents the positions of the samples in the parentheses as all these samples showed the same or very close values.

FIG. 2. Division of tsR20 Filaments at 20°C.

After 120 min of incubation in thymine deficient M9 medium at 42°C, the filaments of tsR20 produced were placed on a glass slide precoated with thymine deficient M9 agar and cultivated at 20°C (time 0). The slide was examined by a phase-contrast microscope at 0, 2, 2.5, 3, 3.5, 4, 4.5, 5.5, and 16 hr of incubation.

at 42°C when the culture was deprived of thymine. The results are presented in Fig. 1.

The filaments of tsR20 produced by 120 min of incubation without thymine at 42°C were placed on a thymine omitted M9 agar-coated glass slide at 20°C and examined under a phase-contrast microscope at different time intervals. As shown in Fig. 2, many of the filaments divided once, twice or three times during 5.5 hr of incubation. Cells which failed to divide or lyzed were also found in the same range of vision. Most cells elongated gradually during incubation. Contrary to tsR20, the parent strain ts20 produced no new cells and only formed filaments longer than tsR20 under the same conditions of cultivation as above. The result coincided with that of total cell count.

From above results of double mutant tsR20, the division of *recA* strain in the absence of DNA synthesis seemed to be accomplished through the process participating in normal division because (1) both divisions were thermosensitive and (2) both divisions after a temperature shift back from 42° to 30°C were sensitive to CP. However, there was no accumulation of division

potential when tsR20 was incubated at 42°C in the absence of thymine in contrast to the presence of thymine. This may suggest that normal synthesis of DNA is required to retain division activity during incubation at restrictive temperature.

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