A Temperature-sensitive Cell Division Component in a Mutant of *Salmonella typhimurium*

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**SUMMARY**

*Salmonella typhimurium* strain 4a is temperature-sensitive. It grows normally at 25° but stops dividing immediately when shifted to 42°. After the shift cell mass increases at a normal rate and DNA synthesis is unaffected. Viable count remains constant for about 3 h. and then falls off sharply. The filaments formed at 42° divide when returned to 25° after a delay which in broth is about 70 min. This delay is not markedly dependent on the time previously spent at 42° but is medium-dependent, being shorter in enriched media. Eventually all the divisions prevented at 42° take place at 25°. Chloramphenicol almost completely stops filament septation and division, and when added early in the recovery period causes fragility, some lysis and a sharp fall in viable count. Nalidixic acid does not stop filament division. It is concluded that a shift to 42° influences cell division directly by causing the irreversible inactivation of a component required at a late stage of division. The length of the division recovery period when filaments are returned to 25° suggests that the component must accumulate throughout most of the division cycle before division can restart.

**INTRODUCTION**

The position of the bacterial septum and the timing of its formation clearly play a critical role in bacterial growth since it must segregate the newly formed chromosome copies and divide the cell substance. Septum formation is precisely regulated since (a) few enucleated bacteria are formed under normal conditions (Hirota, Jacob, Ryter, Buttin & Nakai, 1968) and (b) newborn bacteria have similar cell sizes (Marr, Painter & Nilson, 1969). It has been recently claimed that the termination of a round of DNA replication triggers cell division, there being at relatively fast growth rates a constant time interval (the D period) between termination and division (Clark, 1968; Helmstetter & Pierucci, 1968). Such triggering of cell division clearly facilitates accurate nuclear segregation, and we have recently argued (Spratt & Rowbury, 1971) that the behaviour of a number of bacterial DNA synthesis mutants can be explained by termination triggering division.

The complete sequence of events or the accumulation of all intermediates necessary for cell division might take place only during the D period (i.e. triggered by termination) since in rapidly growing bacteria the mean generation time is approximately equal to D min. (Helmstetter & Pierucci, 1968). There might, however, be cell division components which are formed continuously, building up between divisions and being used up in the division process. Smith & Pardee (1970) have shown that heat-induced cell division synchronization of *Escherichia coli* Hfr is dependent on the inactivation of a heat-labile protein which is formed throughout the division cycle.

We have isolated a temperature-sensitive mutant of *Salmonella typhimurium* (strain 4a)
which forms filaments at 42°. Its cell division behaviour on transfer from 25° to 42° and back to 25° suggests that the lesion is in a division component required in a terminal stage of cell division but formed (or at least present in a temperature-sensitive state) during most of the division cycle and at a rate dependent on the growth rate.

METHODS

Organisms. Strain 4α derives from a histidine-requiring strain (his-2253, his-T1504) of Salmonella typhimurium provided by Dr R. G. Martin. Organisms of the parent strain grown in nutrient broth were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine by the method of Adelberg, Mandel & Chen (1965). After mutagenesis organisms were washed free of mutagen, allowed to grow for several hours in broth and then plated on nutrient agar (NA) at 25°. Strain 4α was one of 20 temperature-sensitive mutants purified from colonies which failed to grow when replicated to 42°.

Media. We have used Oxoid nutrient broth no. 2. Nutrient agar (NA) contained this medium solidified with 2% Difco Bacto agar. The minimal medium (MM) was that of Davis & Mingioli (1950) with 0.2% glucose and 30 μg./ml. DL-histidine. For Casamino MM this medium was enriched with 0.1% Difco vitamin-free Casamino acids.

Growth of organisms. Organisms were subcultured from a nutrient agar slope to the appropriate liquid medium, incubated overnight in a shaking water bath, diluted and grown for 1½ to 2½ h. to obtain an exponentially growing culture. Turbidity of cultures was measured in the Hilger photoelectric colorimeter against an uninoculated medium blank (filter 49 for minimal media and filter 52 for nutrient media). For viable counts cultures were diluted in 0.065 M phosphate buffer, pH 7.4, and appropriate dilutions were plated in triplicate on NA plates. The plates were incubated for 40 h. at 25°. Total cell number was measured using a Coulter model F electronic particle counter. Samples were diluted into Isoton diluent (Coulter Electronics Ltd, Dunstable, Bedfordshire) and 0.1 ml. counted with the 30 μm. orifice with a threshold of 4, attenuation 1 and aperture 8.

To study cell division on agar we diluted cells growing exponentially in nutrient broth and poured them on to a thin film of NA. The seeded film of agar was transferred to a clean microscope slide, surrounded with blocks of NA and covered with a cover-slip to keep it moist. The preparation was examined at 15 min. intervals under a phase contrast microscope.

Synthesis of DNA. DNA synthesis was measured by the incorporation of labelled thymidine into cold trichloracetic acid-insoluble material in the presence of uridine. Organisms growing exponentially in broth + uridine (1.5 mM) at 25° were diluted into fresh broth + [3H]-thymidine (0.1 μCi/ml.) + uridine (1.5 mM) and incubated at 42°. Samples (1 ml.) were removed at intervals into an equal volume of ice-cold 10% trichloracetic acid (TCA) containing 40 μg./ml. unlabelled thymidine. Samples were then treated as described previously (Spratt & Rowbury, 1970).

Chemicals. Methyl-[3H]thymidine obtained from The Radiochemical Centre, Amersham, Buckinghamshire, was supplied at 50 Ci/mm. Chloramphenicol was obtained from Parke, Davis & Co., Hounslow, Middlesex. All other chemicals were of at least analytical grade in purity.
RESULTS

Inhibition of cell division at the restrictive temperature

When a culture of *Salmonella typhimurium* strain 4a growing exponentially in nutrient broth medium at 25°C is shifted to 42°C in the same medium, cell division, as measured by viable counts (Fig. 1), or Coulter counts (data not shown), stops immediately or almost immediately. Increase in cell mass continues quite normally for some hours so that long filaments are formed. After the first hour at 42°C no normal-sized cells are observed. Viability begins to decrease sharply after about 3 h. (Fig. 1).

Fig. 1. Growth and cell division of *Salmonella typhimurium* strain 4a at 42°C. Organisms growing exponentially at 25°C in nutrient broth were diluted with the same medium at time zero and incubated at 42°C with shaking. Samples were removed at intervals of 30 min. for measurements of viable counts and turbidity (see Methods). ○, Viable counts; □, dry wt of organisms determined from an extinction/dry wt curve.

Fig. 2. Growth and cell division of strain 4a at 37°C. Organisms growing exponentially at 25°C in nutrient broth were diluted with the same medium at time zero and incubated at 37°C. Samples were removed at the stated intervals for measurements of total cell number (using the Coulter counter—see Methods) and turbidity. ○, Dry wt; ▼, total cell number.

Strain 4a will not form colonies at 37°C. However, when organisms growing at 25°C in liquid medium are shifted to 37°C extensive division does occur. On NA at 37°C organisms elongate for a period to form filaments which then divide both medianally and terminally. All the daughter cells go on to form filaments. Almost all the original cells divide at least once and most divide twice during the experimental period (2½ h.). Very similar behaviour occurs in nutrient broth at 37°C (Fig. 2). Cell mass increases without a marked rise in cell count for the first ¾ h. There is then a burst of division. Later increases in cell count more or less parallel the mass increase.

Since division stops immediately at 42°C it is evident that the component altered in strain 4a must be involved in a late stage of division. At 37°C some of the component must remain active long enough for it to be used for division. The fact that all the cells produced by
division at 37° go on to form filaments and therefore eventually lose viability may explain the failure of 4a to form even small colonies at 37°.

**DNA synthesis in strain 4a at 42°**

Inhibition of DNA synthesis leads eventually to cessation of cell division (Helmstetter & Pierucci, 1968) and in a few cases the effect is immediate (Donachie, 1969). Since the failure of strain 4a to divide at 42° might therefore be secondary to such an effect the rates of DNA synthesis (as measured by incorporation of thymidine into cold TCA-insoluble material in the presence of uridine) were followed at 42° in strain 4a and in its parent. Over a period of 1½ h. (Fig. 3a, b), the rates were virtually identical.

![Graph](image)

**Effect of salt on division at 42°**

Certain division mutants of *Escherichia coli* divide at the restrictive temperature if sodium chloride is added, and the salt appears to correct membrane lesions which are present (Ricard & Hirota, 1969; Reeve, Groves & Clark, 1970). Added salt (to give a final concentration of 11 g./l. of nutrient broth) did not induce division in cultures of strain 4a which had been at 42° for 1 h. (Fig. 4). This suggests that strain 4a may have normal membrane function and the insensitivity of the strain at 42° to lysis by deoxycholate (which frequently lyses cells with membrane lesions: De Zwaag & Luria, 1967; Hirota, Mordoh & Jacob, 1970) is compatible with such an interpretation.

**Recovery of cell division ability after a shift from 42° to 25°**

A number of filament-forming mutants restart division shortly after return to the permissive temperature, indicating that inactivation of the altered component is reversible or that synthesis of a sufficient quantity for division takes only a short time. To obtain similar information about strain 4a, filaments formed at 42° were returned to 25° and examined at
A division mutant of Salmonella typhimurium

intervals. Microscopic examination showed that 50 to 60 min. elapse before septa are visible and there is a further 10 to 15 min. before normal-sized cells are formed. Measurement of viable counts (Fig. 5) confirm that division begins at 60–70 min. after the return to 25°C. The numerous divisions prevented at 42°C then take place at a rate faster than cell mass increases (Fig. 5) and eventually all the divisions delayed at 42°C take place (Table 1). Shifting to 42°C during the recovery period stops the division process (Fig. 5). Direct observation of filaments on NA confirms that complete septation occurs at 25°C and shows that the initial divisions appear to occur randomly throughout the filaments.

The length of the recovery period at 25°C before division restarts is largely independent of the time previously spent at 42°C; it is slightly less after short incubations (Fig. 5 and 6).

![Graph](image)

Fig. 4. The effect of sodium chloride on cell division. Organisms of strain 4a growing exponentially in nutrient broth at 25°C were shifted to 42°C at time zero. At 1 h. (J) the culture was divided and NaCl (6 g./l. to give final concentration of 11 g./l.) was added to one portion. Incubation was continued for a further 2 h. at 42°C. Extinction (from which cell mass in mg. dry wt/ml. was calculated) and total cell counts (by Coulter counter) were measured at the stated intervals. Control culture (no NaCl added): O, total cell counts; ●, cell mass. NaCl-treated culture: □, total cell counts; ■, cell mass.

| Table 1. Division of Salmonella typhimurium strain 4a at 25°C after incubation at 42°C |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Initial viable count | Final viable count | Initial viable count | Final viable count |
| Period at 42°C                 | At 42°C | At 25°C | Total | At 42°C | At 25°C | Total |
| 40 min.                        | 1.0     | 6.7     | 6.7   | 2.0     | 2.9     | 5.8   |
| 75 min.                        | 1.0     | 32.0    | 32.0  | 3.5     | 10.0    | 35.0  |
| 150 min.                       | 1.1     | 50.0    | 55.0  | 9.0     | 5.0     | 45.0  |
It is evident, therefore, that inactivation of the temperature-sensitive division component of strain 4a is irreversible and that it must be synthesized for a substantial period at 25° before division can restart. The effect of shifting to 42° during recovery makes it probable that synthesis of the component must occur throughout the recovery period.

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**Fig. 5**
Division of strain 4a at 25° after a period at 42°. Organisms growing exponentially in nutrient broth at 25° were shifted to 42° for 75 min. The culture was then diluted with the same medium and incubated at 25°. Samples were taken at the time of the return to 25° (zero) and at 30 min. intervals thereafter. ○, Viable count; □, dry wt of organisms. At 50 min. after the shift to 25° a portion of the culture was returned to 42° for the duration of the experiment. ●, Viable count; ■, dry weight.

**Fig. 6**
Cell division of strain 4a at 25° after various periods at 42°. Organisms growing exponentially at 25° in nutrient broth were shifted to 42° for 40 min. (□) or 120 min. (○). Cultures were then diluted with broth and incubated at 25°. Samples for viable counts were taken at the time of the return to 25° (zero) and at the stated intervals.

**The effects of chloramphenicol and nalidixic acid on cell division recovery**

In some septation mutants (Hirota, Ryter & Jacob, 1968; Reeve et al. 1970) recovery at the permissive temperature can occur even when all protein synthesis is inhibited with high levels of chloramphenicol. Filaments of strain 4a do not, however, divide at all if chloramphenicol (CAP) is added at 25°. Direct microscopic examination shows that almost no septation occurs if CAP (200 μg./ml.) is added at the time of the return to 25° or 30 or 60 min. later. Fig. 7 shows that there is only a small increase in viable count when CAP is added 60 min. after the return of filaments to 25° (i.e. 5 to 10 min. before division begins) while when added earlier there is a sharp fall in viability apparently due to fragility since extinction measurements (data not shown) suggest that lysis occurs.

These results confirm that the inactivation of the temperature-sensitive component is irreversible (since protein synthesis is essential for its reformation) and imply that it is a protein which is formed de novo.

If the DNA formed by strain 4a at 42° is normal and its segregation precise then inhibition of DNA synthesis at the time of the shift back to 25° should not prevent division. This was confirmed by using the DNA synthesis inhibitor nalidixic acid. At 15 μg./ml. inhibitor the extent of division of filaments shifted back to 25° was approximately that expected from the increase in cell mass which had occurred at 42° (data not shown).
Fig. 7. The effect of chloramphenicol on the division of filaments at 25°C. Organisms of strain 4a growing exponentially in nutrient broth at 25°C were shifted to 42°C for 75 min. After dilution with broth, cultures were shifted to 25°C (time zero) and samples taken for viable counts at the stated intervals. Chloramphenicol (200 μg./mL.) was added at time zero (△), at 30 min. (○), at 60 min. (□) or not at all (○).

Table 2. Effect of the growth medium on the length of the cell division recovery period

Organisms growing exponentially in the stated medium at 25°C were diluted into the same medium and incubated at 42°C for the stated time. After further dilution incubation was continued at 25°C for 2½ to 4 h. depending on medium. Turbidity measurements and viable counts were followed throughout as described in Methods. Cell division recovery time is the time at 25°C before division begins. Time for delayed divisions is the time taken (once division has begun) for the viable count to increase to an extent equivalent to the turbidity increase at 42°C.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Time at 42°C</th>
<th>Initial turbidity at 42°C</th>
<th>Cell division recovery time at 25°C</th>
<th>Time for delayed division at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>75 min.</td>
<td>3.1</td>
<td>ca. 75 min.</td>
<td>ca. 30 min.</td>
</tr>
<tr>
<td>Casamino MM</td>
<td>120 min.</td>
<td>3.0</td>
<td>ca. 105 min.</td>
<td>ca. 60 min.</td>
</tr>
<tr>
<td>Glucose MM + histidine</td>
<td>150 min.</td>
<td>2.7</td>
<td>ca. 120 min.</td>
<td>ca. 90 min.</td>
</tr>
</tbody>
</table>

Effect of growth medium on the length of the cell division recovery period

Cell division is triggered by the termination of rounds of DNA replication and follows after an interval (the D period) which is relatively constant at fast growth rates (Helmstetter & Pierucci, 1968). It was possible that strain 4a was altered in a D period component. In such a case (provided that the events of the D period are reversible) division after a period at 42°C should begin not more than D min. after the return to 25°C. Since the D period should
not be appreciably longer in MM or Casamino MM than in broth (Helmstetter, Cooper, Pierucci & Revelas, 1968) the cell division recovery period should be the same in all three media. In fact the recovery time depends on the medium used. Thus after a period at 42° the recovery time is extended from about 75 min. in nutrient broth to 105 min. in Casamino MM and to 120 min. in MM + histidine (Table 2). Once division begins at 25° the time taken for the delayed divisions to occur is also medium-dependent (Table 2). Thus unless the D period at 25° is greatly extended in the poorer media a lesion in a D period component can be ruled out.

**DISCUSSION**

The lesion in strain 4a appears to affect the cell division process itself and not to be one which affects DNA synthesis primarily and cell division only indirectly, since DNA synthesis continues normally at 42° in strain 4a and division resumes at 25° in the presence of nalidixic acid after a period at 42°. The complete septation of filaments incubated at 25° (Table 1) probably indicates that the segregation of the nuclear bodies formed at 42° is also normal. The irreversibility of the lesion of strain 4a by salt and the insensitivity of the strain to deoxycholate suggest that membrane function is also normal.

It is clear that the lesion in strain 4a is in a late division stage since even cells which are about to divide are prevented by a shift to 42°. Unlike other mutants with this characteristic (Hirota et al. 1968; Reeve et al. 1970) recovery is not rapid. It is evident that the temperature-sensitive component (S) is present or is being formed during most of the period between divisions since recovery takes a substantial part of a generation time in the media tested and can be prevented by returning the filaments to 42° during the recovery period. Three explanations seem possible. First, S might be formed early in the division cycle in a burst of synthesis and then used for division via a sequence of heat-labile intermediates. Secondly, after formation early in the cycle, S might remain in a temperature-sensitive state until used up in a terminal division stage. In the interim between formation and utilization other essential processes would occur. To explain the length of the recovery period it would be necessary for these processes to be prevented at 42°. Thirdly, S might be synthesized continuously throughout growth, division occurring when a critical amount has accumulated, this being then used up in the division process. This latter possibility seems the most attractive since it does not assume that the restrictive temperature arrests any process except the formation of S. The inhibition of recovery by chloramphenicol is compatible with such an accumulation process while the dependence of the recovery time on the growth rate implies that S might be formed as a constant fraction of the total protein. In many respects the temperature-sensitive component of strain 4a behaves like the component studied by Smith & Pardee (1970).

**REFERENCES**


Clark, D. J. (1968). The regulation of DNA replication and cell division in *E. coli* b/r. *Cold Spring Harbor Symposia on Quantitative Biology* 33, 823-838.


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