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Unlinking of Cell Division from Deoxyribonucleic Acid Replication in a Temperature-sensitive Deoxyribonucleic Acid Synthesis Mutant of *Escherichia coli*

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A new type of temperature-sensitive deoxyribonucleic acid (DNA) synthesis mutant, which can divide without a completion of DNA replication, was isolated from a thymidine-requiring *Escherichia coli* strain by means of photo-bromouracil selection after nitrosoguanidine mutagenesis. In this mutant, in spite of the fact that DNA synthesis stopped immediately after the temperature shift from 30 to 41 C, cells could continue to divide, though at a reduced rate. This cell division without DNA synthesis at 41 C is further supported by the following results. (i) Cell division took place at high temperature without addition of thymidine but not at all at 30 C. The parent strain of the mutant did not divide at 41 C without thymidine. (ii) Smaller cells isolated from the culture grown at 41 C did not contain DNA. This was shown by chemical analysis of the smaller cells and on electron micrographs. Ability of cells to divide was examined according to sizes of cells. By using the culture at 30 C, cells of various sizes were separated by means of sucrose-density gradient centrifugation. It was found that all cell fractions, including the smallest one, could divide at high temperature. These results suggest that in this mutant the completion of DNA replication is not required for triggering cell division at high temperature. Heat sensitivity of a factor which links cell division with DNA replication appears to be responsible. Some possible mechanisms of the coordination between cell division and DNA replication are discussed.

Cell division is thought to be coordinated strictly with deoxyribonucleic acid (DNA) replication. This is supported by the following facts. When DNA replication in normal cells is stopped by thymine starvation, cell division does not take place, and cells only elongate without septation. This ceasing of cell division upon blocking DNA replication is also a common feature for the various kinds of temperature-sensitive DNA synthesis mutants of bacteria so far reported (3, 9, 12, 15, 18). In the case of a mutant in which DNA synthesis continues for a while after the temperature shift and then stops, cell division occurs even after the end of DNA synthesis, so that bacteria lacking DNA are produced (12). However, if DNA synthesis is blocked by means of thymine starvation or nalidixic acid at the time of the temperature shift, no cell division is observed, suggesting that the completion of DNA replication is required for triggering cell division (12). The requirement of the completion of DNA replication for cell division was also shown by Helmstatter and Pierucci (11) and Clark (6).

The present paper deals with a new type of temperature-sensitive DNA synthesis mutant of *Escherichia coli* in which the completion of DNA replication is not required for cell division at high temperature. DNA synthesis of this mutant stops immediately after a temperature shift, but the cells keep dividing. When DNA synthesis is blocked by thymidine starvation, no cell division is observed at 30 C, but cells can divide at 41 C, although the rate of cell division is reduced and the sizes of cells produced are not uniform. The fact is confirmed by isolating cells lacking DNA from the culture at 41 C. Furthermore, it was found that even newborn cells at 30 C can divide at 41 C, although the rate of cell division is reduced and the sizes of cells produced are not uniform. The fact is confirmed by isolating cells lacking DNA from the culture at 41 C. Further-

more, it was found that even newborn cells at 30 C can divide at 41 C as well as other cell fractions, suggesting that the completion of DNA replication is not required for cell division at the high temperature. From these results, and from some other properties of the mutant, triggering of cell division under normal conditions is suggested to be controlled negatively by a factor which is thermolabile in the present mutant. Thus a mechanism which requires the completion of DNA replication for normal cell division is discussed.

MATERIALS AND METHODS

Isolation of temperature-sensitive DNA synthesis mutants. To select temperature-sensitive DNA synthesis mutants, 5-bromouracil was used to kill normal cells, since only normal cells which can incorporate 5-bromouracil into their DNA at high temperature are killed by longer wavelength ultraviolet irradiation (4; R. Eisenberg and A. B. Pardee, *submitted for publication*). The procedure is as follows: By using *E. coli* MX74 Sm^r Lac^- F^-, a thymidine auxotroph, *E. coli* MX74 T2 which could grow in the presence of 3 μg of thymidine per ml was isolated with use of trimethoprim, by the method of Dubnau and Mass (8). *E. coli* MX74 T2 was then mutagenized by treating cells with 1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml in 0.2 N acetate buffer (*p*H 5.0) for 30 min at 37 C. After the treatment, the cells were washed and grown at 30 C in M9 medium (7 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, and 0.2 g of $MgSO_4$/liter of water) supplemented with 4 mg of glucose per ml, 2 μg of vitamin B_1 per ml, 2 mg of Casamino Acids (Difco) per ml, and 3 μg of thymidine per ml. Exponentially growing cells were then diluted with the same medium to about 2×10^8 cells/ml, and they ere incubated at 41 C for 30 min. A 5-ml amount of the culture was added to 15 ml of M9 medium at 41 C, supplemented as described above but lacking thymidine; 5-bromouracil was added to the mixture to a final concentration of 20 μg/ml. The mixture was incubated at 41 C for 1 hr. After incubation, 10 ml was filtered and the cells were resuspended in 30 ml of cold M9 medium without any supplement. A 15-ml amount of the suspension was irradiated with 313 nm ultraviolet light according to Eisenberg and Pardee (*submitted for publication*). The fraction of survivors of this treatment was 3×10^{-3}. Cells were then grown at 30 C overnight in Penassay Broth and plated on Penassay plates which were incubated at 35 C. Since temperature-sensitive mutants should grow slowly at this intermediate temperature, they form much smaller colonies than normal cells. Thus, after overnight incubation of the plates, small colonies were picked and their temperature sensitivities were checked. In this way one-third to two-thirds of the picked colonies were found to be temperature-sensitive.

For the selection of temperature-sensitive DNA synthesis mutants, cells were incubated in Penassay Broth at 30 C until early exponential phase. Then the temperature was shifted to 41 C, and incubation was continued for 3 to 4 hr. Each culture was then examined microscopically; for those which contained long cells, DNA synthesis was examined by ^{3}H-thymidine incorporation at 41 C.

DNA synthesis. DNA synthesis was asayed by the incorporation of ^{3}H-thymidine into DNA in a way previously described for amino acid incorporation (22). An 0.5-ml amount of culture in M9 medium supplemented with glucose, vitamin B_1, and thymidine was added to 1 ml of M9 supplemented with glucose, B1, 3 μg of thymidine per ml, and 10 μc of thymidine-*methyl*-^{3}*H*, and the mixture was incubated at 30 or 41 C. At time intervals, 0.1 ml of the mixture was taken onto a circular filter paper (Whatman 3MM, 2.4-cm diameter), which had been presoaked in 5% trichloroacetic acid containing 50 μg of thymidine per ml and dried. Just after the sample spread over all the paper, it was put into a cold 5% trichloroacetic acid solution containing 50 μg of thymidine for about 1 hr. The paper was washed twice by changing the trichloroacetic acid solution, soaked in acetone, and dried. Radioactivity was determined in a liquid scintillation counter.

Chemical analysis. DNA content was determined by a modified diphenylamine method (10). Protein was determined by the method of Lowry et al. (17).

Enzyme assay. DNA polymerase was measured by the method of Lehman (16) with the fraction partially purified by streptomycin.

β-Galactosidase was measured by *o*-nitrophenyl β-D-galactopyranoside hydrolysis by bacteria treated with toluene (21).

Separation of smaller cells from longer cells (snakes) was by means of sucrose-density gradient (5 to 30%) centrifugation as described by Mitchison and Vincent (19).

Counting of cell number. Cell number was counted microscopically with use of a Petroff-Hausser and Helber counting chamber. Photographs were taken with a Zeiss Ultraphotomicroscope (Nomarski optics).

Observation of DNA in the cells. DNA in the cells was observed with the electron microscope by the method of Peters and Wigand (20).

Bacteriophages. Bacteriophage T4 (wild type) and λ ref were used. In the case of T4 phage, two cultures of *E. coli* MX74 T2 ts27 were preincubated for 40 min at 30 and 41 C, respectively, and then they were infected with T4 phage. Incubation was continued for 45 min at 30 and 41 C, respectively. Burst sizes were measured as described by Adams (1). In the case of λ phage, burst sizes were determined at 30 and 41 C without preincubation, by the method of Kaiser and Hogness (14).

Bacterial mating. To obtain *E. coli* MX74 T2 ts27/F' Lac^+, *E. coli* MX74 T2 ts27 was mated with *E. coli* RV Sm^s Lac^-/F' Lac^+. Each parent was grown to about 10^8 cells/ml at 30 C in Penassay Broth. *E. coli* MX74 72 ts27 and RV/F' Lac^+ were mixed at a ratio of 1 to 10, and the mixture was incubated at 30 C for 45 min. The mating product was picked up from a MacConkey plate containing 150 μg of streptomycin per ml.

RESULTS

Isolation and characterization of a temperature-sensitive DNA synthesis mutant. The mutants which produced long cells (snakes) at high temperature were isolated as described and tested for rates of thymidine incorporation at 41 C. They were classified into two groups. In the first group, DNA synthesis stopped immediately after the temperature shift, and in the second group it continued for about 1 hr before it finally stopped. In the former group, one interesting mutant, *E. coli* MX74 T2 ts27, was found. This mutant could grow as well as the wild-type *E. coli* MX74 T2 at 30 C in minimal medium supplemented only with thymidine. However, at 41 C, DNA synthesis stopped immediately (Fig. 1), and snakes were produced (*see* Fig. 3). It should be noticed that DNA synthesis which stopped at 41 C could resume without any lag even after 1 hr of incubation at 41 C when the culture was put back to 30 C (Fig. 1b). The rate of DNA synthesis after the shift back to 30 C is higher than normal, until it catches up to the normal DNA content. An immediate stop of DNA synthesis was shown not only by ^{3}H-thymidine incorporation (Fig. 1) but also by measuring total DNA content (Fig. 2), which did not increase at 41 C, whereas optical density and protein content increased exponentially.

Cell division at high temperature. In spite of an immediate stop of DNA synthesis at 41 C, total cell numbers increased 2.5 times during a period of several hours, although the rate of cell division was about one-third of normal (Fig. 2). After 3 hr of incubation at 41 C, DNA content, protein content, optical density, and cell number increased 1.1, 5.1, 6.4, and 2.5 times, respectively. Thus snakes were formed at high temperature because of the slow rate of cell division relative to the faster increase of mass. Snakes formed by 4 hr of incubation at 41 C and cells grown at 30 C are shown in Fig. 3; they are more than 10 times as long as normal cells. Smaller cells can be seen together with the snakes; their lengths were not uniform and varied from about one-half to two or three times the size of normal cells. Corresponding to various lengths of smaller cells, abnormal septations occurred at one end of a

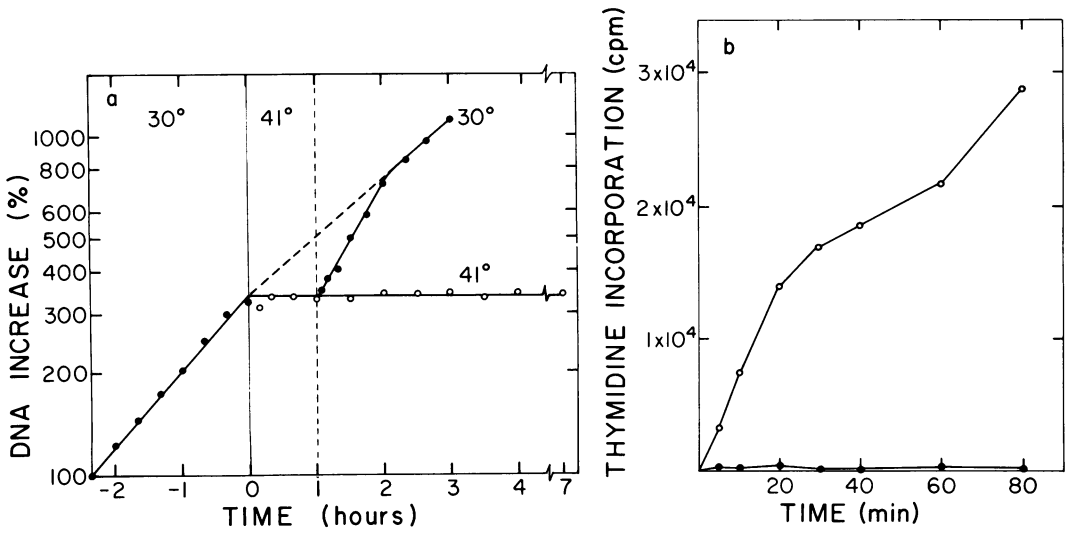

FIG. 1. *^{3}H-thymidine incorporation of E. coli MX74 T2 ts27 at 41 C. (a) A 1-ml amount of M9 medium supplemented with glucose, vitamin B_1, $MgSO_4$, and Casamino Acids containing 10 μc of thymidine-methyl-^{3}H was preincubated at 41 C. A 0.5-ml amount of the culture grown at 30 C in M9 supplemented with glucose, vitamin B_1, $MgSO_4$, and Casamino Acids containing 3 μg of cold thymidine per ml was added. At time intervals indicated, 0.1 ml of the mixture was taken onto filter paper. Wild-type strain E. coli MX74 T2 (○); mutant E. coli MX74 T2 ts27 (●). Cell concentrations of the final mixtures were 4 × 10^7 cells/ml. (b) Into 4 ml of M9 supplemented with glucose, vitamin B_1, $MgSO_4$, 3 μg of cold thymidine per ml, and 40 μc of ^{3}H-thymidine, 1.5 ml of a culture of E. coli MX74 T2 ts27 grown at 30 C in M9 supplemented as above, except without ^{3}H-thymidine, was added. The mixture was incubated at 30 C for 140 min; the temperature was then shifted to 41 C. After 1 hr of incubation at 41 C, 2 ml of the mixture was again returned to a 30 C water bath. At time intervals indicated, 0.1 ml of the mixture was removed, and measurement of ^{3}H-thymidine incorporation was carried out as described. The values are expressed as relative increments on the basis of DNA at the starting point (140 min before the temperature shift), which was calculated with use of incorporated radioactivity and the generation time obtained by the change of optical density. ^{3}H-thymidine incorporation at 30 C (●), and at 41 C (○).*

cell, but the position of septum formation was not constant, so that smaller cells of various sizes were produced. In Fig. 4b and 4c, very small cells are shown, and the upper right arrow in Fig. 4a shows an almost normal-sized cell. Formation of two septa at one end of a snake was often observed (two arrows, Fig. 4a and c); in some cases septation occurred centrally (Fig. 4d).

Viability of the cells changed after temperature shift. Portions of the culture were taken at time intervals (Fig. 5), diluted, and plated on Penassay plates which were incubated at 30 C overnight. Since the plating efficiency at zero time was always low (about 70%), *E. coli* B/r was plated at the same time as a reference to obtain an exact correlation between viable count and total cell number obtained microscopically. The viable count increased rapidly after the temperature shift and at 30 min it reached a little above 100% of the initial cell number (Fig. 5). This value was maintained for about 1 hr and then began to decrease.

DNA content of smaller cells. The fact that cells divide without DNA synthesis indicates that some cells have no DNA. A separation by means of sucrose-density gradient centrifugation (19) of smaller cells from snakes was carried out on cultures of the mutant grown at 41 C for 5.5 and 4 hr, respectively. The contents of DNA and protein were measured for the fraction of the smaller cells, the snakes, and also of the cells grown at 30 C. The DNA content of the fraction of the smaller cells was only 5 and 12% of that of cells grown at 30 C for the 5.5-hr culture and the 4-hr culture, respectively, although there was no difference in protein content on the basis of cell number (Table 1). This suggested that the majority of the smaller cells in the fractions contain no DNA or that all smaller cells contain a small amount of DNA. In the snakes fraction, the content of protein was more than 10 times larger than that in normal cells, whereas DNA content was almost the same as that of the normal cells.

Existence of cells lacking DNA in the culture grown at 41 C for 4 hr was also observed by electron microscopy (Fig. 6). Cells lacking DNA were pinched off from snakes which contained DNA (Fig. 6A). Cells of various sizes lacking DNA are shown in Fig. 6B. Sometimes a tripoled cell, such as shown in Fig. 6C, was observed, although it is not clear whether such cells are produced by budding-off of a part of a cell or by fusion of two cells.

Further evidence for cell division without completion of DNA replication. The completion of DNA replication is thought to be required in normal cells in order to trigger cell division, since, when DNA replication is stopped by thymidine

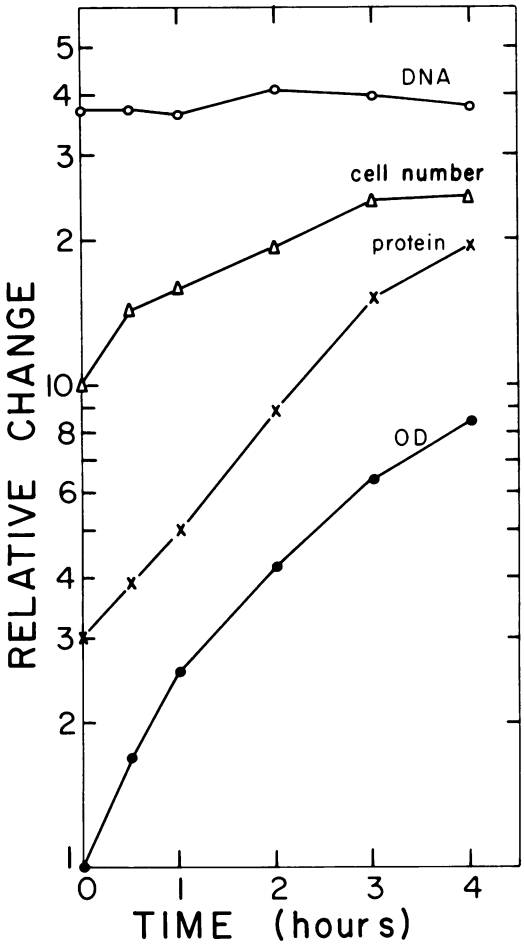

FIG. 2. *Changes of DNA content, protein content, optical density, and cell number after the temperature shift. A culture of E. coli MX74 T2 ts27 which was growing exponentially at 30 C in M9 (glucose, vitamin B_{12}, $MgSO_4$, and 3 μg of thymidine per ml) was transferred to 41 C at zero time. At time intervals indicated, samples were taken and DNA and protein contents were analyzed as described.*

starvation, cell division also stops immediately. This is shown in Fig. 7a and b, which show that cell division stopped immediately after thymidine starvation both with the wild-type strain grown at 41 C and the mutant grown at 30 C. However, the mutant grown at 41 C continued to divide regardless of the presence or absence of thymidine in the medium (Fig. 7c). After 4 hr of incubation at 41 C, cell number increased 2.0- and 1.8-fold with and without thymidine, respectively. In contrast, at 41 C, cell number as well as optical density increased in the absence of thymidine as well as in the presence of thymidine. These results provide further evidence for unlinking of cell

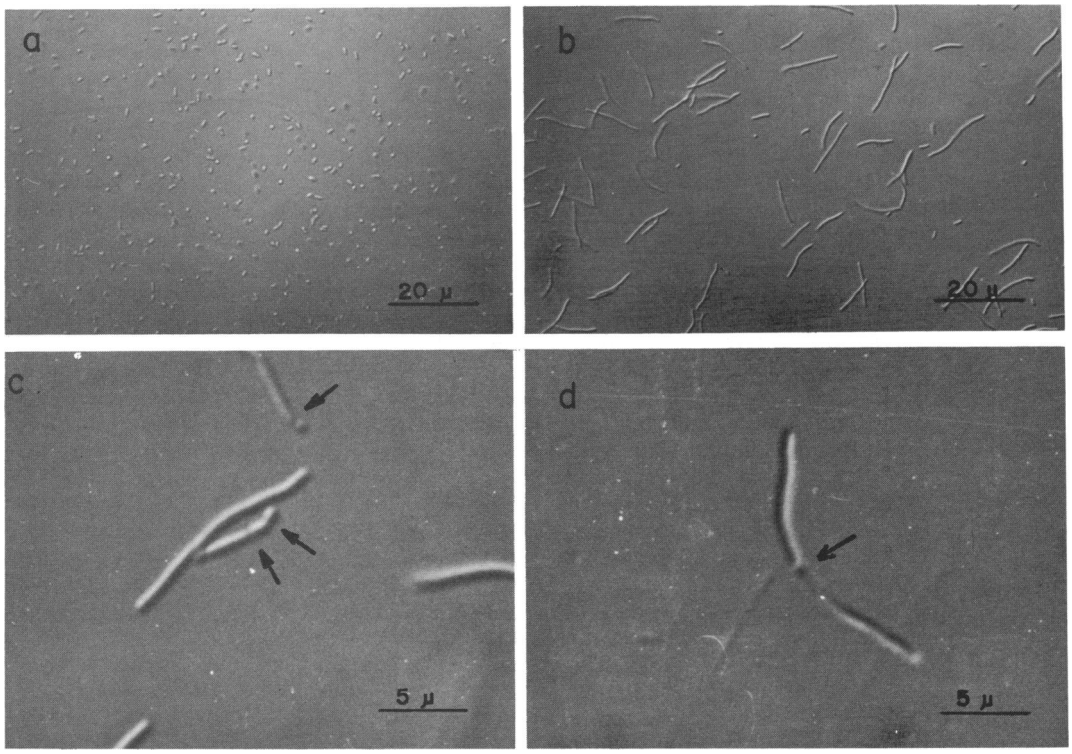

FIG. 3. *E. coli MX74 T2 ts27. (a) At 30 C; (b) after 4 hr of incubation at 41 C.*

division from completion of DNA replication at the higher temperature.

However, when nalidixic acid was used to inhibit DNA synthesis, cells did not divide at 41 or at 30 C (Table 2). At 10 μg of nalidixic acid per ml, cell number increased only 1.29- and 1.19-fold after 4 hr of incubation at 41 and 30 C, respectively, in contrast to 2.16- and 8.10-fold increases to total cell number at 41 and 30 C in the absence of nalidixic acid, respectively. In the presence of 15 μg of nalidixic acid per ml, a strong inhibitory effect on cell division was observed (Table 2). The increase of optical density was not inhibited as much at 41 as at 30 C. These results suggest that nalidixic acid acts not only directly on DNA synthesis but also in some way on the process of septum formation. The results obtained by Boyle, Cook, and Goss (5) for the action of nalidixic acid do not identify the primary target.

The second evidence for cell division without completion of DNA replication at 41 C is as follows. In the culture of the mutant at 30 C, there were cells of various sizes, corresponding to newborn cells (smallest) and cells which are just ready to divide (largest). Since DNA replication of newborn cells is supposed not to be completed (7), ability to divide at 41 C was examined for cells of different sizes. Cells from a culture grown at 30 C were separated by sucrose-density gradient centrifugation (19). After centrifugation, cells were fractionated into five fractions, numbered from the smallest to the largest (Fig. 8). Four of the fractions were then incubated at 41 C for 4 hr, and the increases of cell numbers were measured. The cell number of the smallest cells increased about twofold, just as with the other fractions (Fig. 8). From the fact that the uppermost fraction showed synchrony of cell division with a lag of about one generation time at 30 C, in contrast to the middle fraction (Fig. 8), it was concluded that the fraction consisted of newborn cells. The DNA of these cells should not be completed (7), and yet they divided at high temperature, indicating that in this mutant completion of DNA replication and cell division are dissociated.

Other properties of the mutant. Exponential synthesis of protein after a shift to high temperature has already been shown (Fig. 2). This can also be demonstrated by the inducibility of β-galactosidase. Since the mutant was *lac*$^-$, an F′*lac*$^+$ was transferred into it to produce *E. coli* MX74 T2 ts27/F′Lac$^+$. After it was preincubated for 40 min at 41 C, β-galactosidase was induced by 0.0125 M thiomethylgalactose for 90 min. The ratio of the activity per unit of DNA at 41 C to that

at 30 C was found to be 1.14. This result indicates that the whole procedure of protein synthesis is completely functional in the mutant even at high temperature.

Lack of DNA synthesis at high temperature was not due to inactivation of DNA polymerase, since this enzyme partially purified from a cell-free extract of the mutant was about three times more active at 41 than at 30 C. Nor was the defect in DNA replication restricted to the chromosome, because the mutant carrying an episome, F'*Lac*$^+$ (*E. coli* MX74 T2 ts27/F'Lac$^+$) could not incorporate ^{3}H-thymidine at all at 41 C. Similarly, λ phage were not reproduced in the mutant at 41 C. The burst size at 30 C was 8.5; at 41 C it was 0.5. These results indicate that a heat-labile factor(s) controls not only synthesis of chromosomal DNA but also episomal and λ phage DNA.

However, bacteriophage T4 could multiply in the mutant at 41 C. After 40 min of preincubation at 30 or 41 C, cells were infected with T4 phage at a multiplicity 0.02 and incubated for 45 min at 30 and 41 C, respectively. The burst sizes were calculated to be 294 and 627 at 30 and 41 C, respectively. This indicates that the thermolabile factor(s) is not involved in the reproduction of T4 phage.

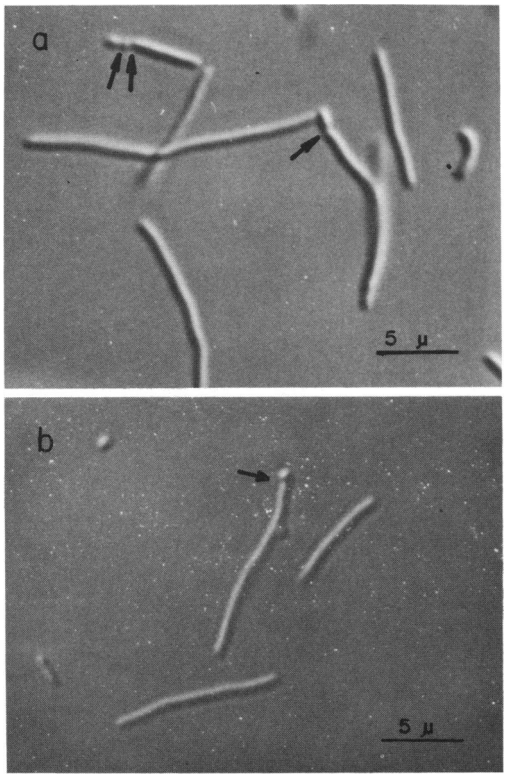

FIG. 4. *Abnormal cell division of E. coli MX74 T2 ts27 grown for 4 hr at 41 C. Arrows show the points where septations are seen.*

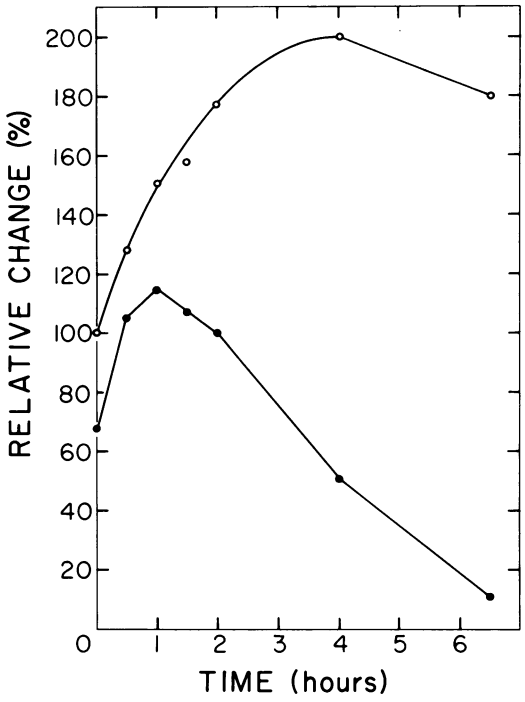

FIG. 5. *Changes of cell number and viability of E. coli MX74 T2 ts27 after the temperature shift. A culture of the mutant grown at 30 C in M9 medium supplemented with glucose, vitamin B_1, $MgSO_4$, and 3 μg of thymidine per ml was transferred to 41 C at zero time. At time intervals indicated, samples were taken for the measurements of total cell number* (○) *and viabile count* (●).

The spontaneous reversion frequency was found to be 2.6×10^{-7}. By using 10 independent revertants, the ability of cell to divide at 41 C in the absence of thymidine was examined. Unlike the temperature-sensitive parent strain, all of these revertants were unable to divide when thymidine was withdrawn from the medium. This suggests that abnormal septum formation and cessation of DNA synthesis at high temperature are caused by a single mutation. This reversion frequency of the mutant is also typical of a single mutation.

DISCUSSION

The mutation reported here makes a part of the DNA synthetic apparatus thermolabile. It does not seem to act at one of the obvious steps of the pathway of DNA or protein synthesis. The entire set of events of protein synthesis required for enzyme production was completely normal at 41 C. DNA polymerase was also fully

active in extracts at 41 C. The rapid reversibility of the inhibition of DNA synthesis upon shifting back to 30 C (Fig. 1) tends to argue against a heat-labile protein or heat-labile protein synthesis. T4 phage could be made at normal burst sizes at 41 C, but replication of λ phage or the F'*lac*$^+$ episome did not occur. This suggests an effect throughout the entire cell, but not one involving host-supplied products required for T4 synthesis.

The mutant in the present study continued to divide at 41 C in spite of an immediate stop of DNA synthesis; DNA-less cells were produced. Completion of DNA replication was previously thought to be required to trigger cell division (6, 11). Thus, if DNA synthesis of wild-type cells is inhibited by thymidine starvation, cells only elongate without division. This is also true of a temperature-sensitive mutant isolated by Hirota et al. (12). This mutant appears to complete synthesis of its chromosome at the nonpermissive temperature, which allows limited division. However, the mutant cannot divide if DNA synthesis is blocked at the time of temperature shift.

That the present mutant divides at high temperature without completion of DNA replication is further shown by the following results. (i) The cell number increased at 41 C regardless of the presence or absence of thymidine (Fig. 7). (ii) Newborn cells divided at 41 C as well as older cells (Fig. 8).

TABLE 1. *DNA and protein contents of smaller cells and snakes of E. coli MX74 T2 ts27 grown at 41 C*

Fraction	DNA (μg/cell)	Protein (μg/cell)
*ts*27 at 30 C	1.20×10^{-8}	6.1×10^{-7}
*ts*27 at 41 C		
Smaller cells (1)[a]	0.06×10^{-8}	6.0×10^{-7}
(2)[b]	0.15×10^{-8}	
Snakes fraction[c]	1.26×10^{-8}	62.8×10^{-7}

[a] Isolated from a culture grown at 41 C for 5.5 hr; 2.2% of the fraction was snakes.

[b] Isolated from a culture grown at 41 C for 4 hr; 3.3% of the fraction was snakes.

[c] Isolated from a culture grown at 41 C for 5.5 hr; 30% of the fraction was normal-sized cells.

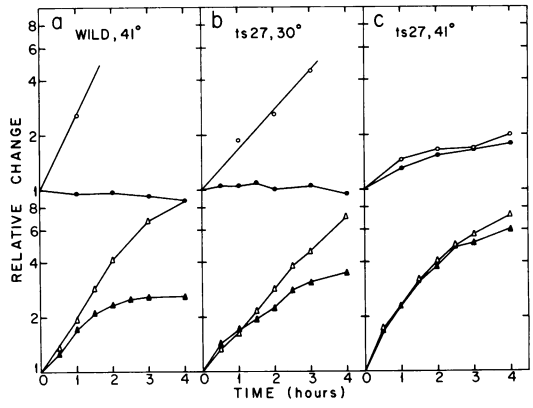

FIG. 7. *Changes of cell numbers and optical densities in the presence and the absence of thymidine. A culture grown at 30 C was centrifuged, and the pellet was resuspended in M9 medium supplemented with glucose, vitamin B_1, and $MgSO_4$. The suspension was separated into two parts; to one of them, thymidine was added to a final concentration of 3 μg/ml. The parts were incubated at 30 or 41 C. (a) Wild-type strain E. coli MX74 T2 at 41 C; (b) E. coli MX74 T2 ts27 at 30 C; and (c) E. coli MX74 T2 ts27 at 41 C. Cell number with thymidine (○), without thymidine (●), optical density with thymidine (△), without thymidine (▲).*

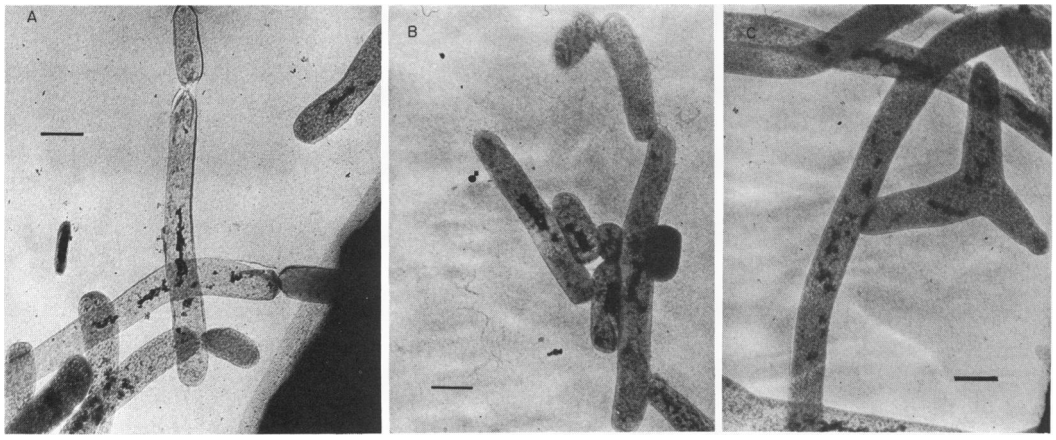

FIG. 6. *Electron micrographs of E. coli MX74 T2 ts 27 grown at 41 C for 4 hr. Nuclear fraction was stained by the method of Peters and Wigand (20). Bars correspond to 1 μm.*

The cessation of DNA synthesis and appearance of septum formation seem to be caused by a single mutation, since the spontaneous reversion frequency agrees with a value for a single mutation, and all independent revertants tested showed simultaneous reversion of both functions. Thus the mutation appears to be pleiotropic, i.e., at the same time that DNA synthetic ability is lost, the cells lose their normal periodic regulatory control of septation. It is possible that there is a second mutation which is dependent on the first and is also required for uncontrolled septation, as described by Hirota et al. (12).

From these results, it is proposed that septum formation in the normal cells is usually blocked by a factor (M), and only at the end of DNA replication is this block suppressed by an unknown mechanism. To trigger septum formation at the end of DNA replication in the normal cells, the septum-formation blocking function of factor M might be inactivated by another unstable factor (I) which is periodically produced in a limited amount at the end of DNA replication. Assume that factor M is simultaneously required for DNA replication; for instance, M factor attaches to an apparatus which strictly coordinates both DNA synthesis and septum formation.

In the present mutant, factor M reversibly dissociates from the apparatus at 41 C, so that septum formation occurs without completion of DNA replication. One could explain the pleiotropic effect of the mutation as follows: DNA synthesis stops and "constitutive" septum formation begins.

The properties of other mutants with deranged DNA-septum interrelations can be accounted for by this model. The minicell-forming mutant (2) might produce excess inducing factor I on completion of DNA replication, so that extra septations occur. The *lon*⁻ mutants stop making septa but continue DNA synthesis after a very low dose of ultraviolet radiation. Possibly ultraviolet radiation causes the M factor to bind firmly on the apparatus. Since reversal of this attachment is not possible in the mutant, DNA synthesis continues but septum formation does not occur. However, there are numerous other possibilities at this stage of knowledge [*see* Witkin (23).] After this paper was submitted, mention of a similar mutant was published by Hirota et al. (13).

TABLE 2. *Effect of nalidixic acid on cell division at 30 and 41 C*

Temp	Hr	Cell no.		Optical density		Cell no.	
		Without nalidixic acid	With nalidixic acid (10 μg/ml)	Without nalidixic acid	With nalidixic acid (10 μg/ml)	Without nalidixic acid	With nalidixic acid (15 μg/ml)
41 C	0	100	100	100	100	100	100
	2					147	100
	4	216	129	540	480	186	112
30 C	0	100	100	100	100		
	2	286	119	283	215		
	4	810	119	755	246		

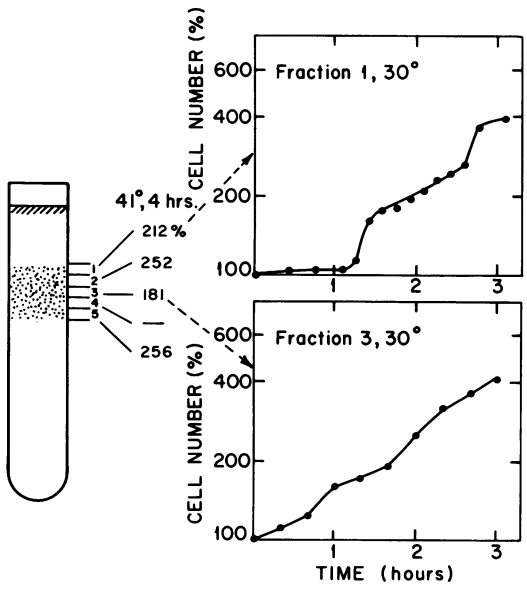

FIG. 8. *Separation of cells by sucrose-density gradient centrifugation. By using a culture of E. coli MX74 T2 ts27 grown at 30 C, cells were separated by sucrose-density gradient centrifugation as shown. They were fractionated into five fractions from the smallest to the largest. Each fraction was then incubated at 41 C for 4 hr in M9 medium supplemented with glucose, vitamin B_1, $MgSO_4$, and 3 μg of thymidine per ml. After incubation, cell numbers were measured. Cell numbers after 4 hr of incubation are expressed as percentages of those at zero time. For fractions 1 and 3, samples were also incubated at 30 C, and, at time intervals indicated, cell numbers were measured.*

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