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Role of the *ftsA* Gene Product in Control of *Escherichia coli* Cell Division

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The kinetics of cell division have been studied in a strain of *Escherichia coli* which has an amber mutation in the *ftsA* gene and which also carries a temperature sensitive amber suppressor. This strain is therefore temperature sensitive for the synthesis of the *ftsA* protein. Cells of this strain were able to divide only if the synthesis of this protein took place during a specific part of the cell cycle. This was a short period (roughly 10 min in duration) immediately before the normal time of cell division.

We have isolated amber (Am) mutations in a number of genes important to the cell cycle in *Escherichia coli* (6; unpublished data). Two of these lie in the *ftsA* gene (6; this paper). We have cloned the wild-type and amber alleles into a phage vector and have shown that the *ftsA* gene codes for a polypeptide with a molecular weight of 50,000 (6). In the present paper we describe the kinetics of cell division in the mutant strains which carry *ftsA*(Am) alleles in combination with a temperature-sensitive (Ts) tRNA [coded for by the *supF-A81*(Ts) allele of the *tyrT* gene] which inserts tyrosine or glutamine at amber codons at the permissive temperature (10). In consequence, these strains are temperature sensitive for the synthesis of the *ftsA* protein. We have used one such strain to find the required time of synthesis of the *ftsA* protein during the cell cycle. We have found that the synthesis of this protein is required during only a short interval just before the normal time of cell division. If it is not made then, the cell cannot divide.

MATERIALS AND METHODS

Bacterial strains. The parental strain from which most other strains have been derived is *E. coli* K-12 "OV2" (4). The genotype of OV2 is as follows: F^- *ilv his leu deo ara*(Am) *lac-125*(Am) *galU42*(Am) *galE trp*(Am) *tsx*(Am) *supF-A81*(Ts). This strain was derived by mutation from strain MB93A81 of Smith et al. (10). It has been used by us in studies on cell growth and division because of its regular and predictable growth and morphology under a variety of conditions (4). OV16 and OV8 are temperature-sensitive strains independently isolated after nitrosoguanidine mutagenesis of OV2 (6). In addition to the mutations carried by OV2, OV16 carries *pro* and *ftsA16*(Am) (6), whereas OV8 carries *gly* and *ftsA8*(Am). Details of the genetic analysis of OV16 and of the identification of the polypeptide coded by the *ftsA* gene have been published earlier (6). The amber mutation in OV8 was shown to be in the *ftsA* gene by complementation tests with the specialized *ftsA* transducing phages described earlier (6). It could also be shown that the amber mutation in the *ftsA* gene in OV8 was at a different site from that in OV16 by marker rescue experiments with λ16-5 [*ftsA16*(Am)].

HB(pLM2) is a strain of *Pseudomonas phaseolicola* carrying the plasmid pLM2 (7). pLM2 is a derivative of RP1 carrying the resistance markers *kan bla*(Am) *tet*(Am) (7). HB(pLM2) was used to donate the plasmid to OV2RR, a spontaneous rifampicin-resistant mutant of OV2. GS100 was a Rif^r Kan^r exconjugant from this cross, which was also temperature sensitive for ampicillin resistance. A λ540 imm^{21} $supF^+$ lysogen of GS100 was made. GS102 was derived by the transfer of pLM2 from HB(pLM2) to *E. coli* KL164 which is a suppressor-free strain having the genotype Hfr *thi-1 relA-1 thyA24 deoB13 nalB14*. GS102 had the phenotype Nal^r Kan^r but was Amp^s at all temperatures. [An additional selection against the donor in crosses involving HB(pLM2) was to carry out the counter-selection at 37°C, a temperature at which *P. phaseolicola* cannot grow (7).]

Phage strain. An integration-proficient specialized transducing phage, λ imm^{21} $supF^+$ derived from λ540 by Borck et al. (2), was used to introduce the non-temperature-sensitive allele of *supF* into recipient strains by lysogenization at 30°C.

Media and growth conditions. Cells were grown in Oxoid nutrient broth no. 2 plus thymine (50 μg/ml) (NBT) with vigorous rotary shaking at 30 or 42°C.

Measurement of cell growth. Growth of cultures was followed by measuring their optical density at 540 nm (OD_{540}). Cell counts and volume distributions were made on samples fixed in 10% formaldehyde with a Coulter Particle Counter (model ZB) with a Coulter

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Channelyzer, as described previously (1). The Channelyzer provides estimates of median cell volume and modal cell volume (in addition to a complete distribution of cell number in each of 100-volume ranges). For most purposes, median cell volume is the more useful parameter, but modal cell volumes give good indications of cell synchrony and are used in some figures. At the time of cell division in synchronous cultures, the volume distribution becomes bimodal.

Photography. Cells were spread on thin layers of agar and photographed under phase-contrast illumination with a Zeiss Ultraphot microscope. For visualization of nucleoids, the agar contained 200 μg of chloramphenicol per ml to cause the DNA to condense (16) and 23% polyvinylpyrollidone to increase the contrast between the nucleoids and the rest of the cell (3).

Estimation of β-lactamase. Samples (2-ml) of log-phase cultures in NBT were frozen and kept overnight at −10°C. The samples were thawed and sonicated (in three 20-s bursts with an MSE sonicator at a 5-μm peak-to-peak amplitude); cell debris was removed by low-speed centrifugation, and the supernatant fluids were assayed. The samples were kept on ice at all stages before assay. Enzyme assay was according to O'Callaghan et al. (9). A 0.5-ml amount of extract was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7) and 20 μl of a freshly made solution (made by dissolving one crystal of the substrate in 0.1 ml dimethyl sulfoxide) of the chromogenic substrate "87/312" added to start the reaction. (The chromogenic cephalosporin substrate 87/312 was a gift from Glaxo Laboratories Limited via Andrew Coulson of this department.) β-Lactamase activity was monitored as increasing OD_{500}. The reactions were carried out at room temperature.

DNA labeling and assay. DNA in OV16 was labeled by growing the cells overnight at 30°C in VB salts (12) plus 0.4% glucose, 0.5% Casamino Acids, 20 μg of tryptophan, 250 μg of deoxyadenosine, and 2 μg of [^{3}H]thymidine (2 μCi) per ml. Such prelabeled cultures were diluted into fresh medium of the same composition, and 50 μl samples were taken at intervals onto 3 MM filter paper disks. The disks were placed in cold 10% trichloroacetic acid and left overnight in the cold, washed three times in cold 5% trichloroacetic acid and once in ethanol, dried, and counted in a liquid scintillation counter.

RESULTS

Effect of temperature shifts on the parent strain OV2. The cell division mutant OV16 was derived by mutagenesis of strain OV2 (6). When cultures of OV2 in log-phase growth in NBT at 30°C were shifted to 42°C, there was a transient slowing down of cell division relative to cell growth during the first 20 min or so. Median cell volume consequently increased by about 25% during this period. However, cell division then took place more rapidly than growth for the next 15 to 20 min, so that median cell volume returned to its initial value. This effect was small but reproducible. No such differential effect on cell division versus cell growth took place when the culture was returned to 30°C.

Kinetics of suppression during temperature shifts in *supF-A81*(Ts) cells. Smith et al. (10) have shown that *supF-A81*(Ts) cells are able to translate about 13% of amber codons at 30°C, but this level drops to less than 1% at 42°C. They were also able to show that the level of suppression drops very rapidly when cells containing the temperature-sensitive tRNA are shifted to 42°C (10). Strain OV2 carries an amber mutation in the *trp* operon, in combination with this temperature-sensitive suppressor. We have measured the kinetics of cell growth in the presence or absence of exogenous tryptophan in this strain at 30°C and after a shift to 42°C. During growth at 30°C, growth was stimulated by added tryptophan, suggesting that the degree of suppression even at the "permissive" temperature is less than adequate to allow normal endogenous tryptophan production. Immediately after a shift to 42°C (in the absence of added tryptophan) cell growth ceased to be exponential and became linear (data not shown). The addition of tryptophan to the medium at this time restored exponential growth. This behavior is consistent with the immediate cessation of the synthesis of tryptophan synthetase when cells were shifted to 42°C and suggests, in confirmation of the results of Smith et al. (10), that suppression drops to a very low level within a few minutes of a shift to 42°C. In the interpretation of the experiments with strain OV16, to be described below, it is also important to know the kinetics of the restoration of suppression when cells which have been growing at 42°C are returned to 30°C. To find this out, we have measured β-lactamase activity in strain GS100, which is a derivative of OV2 carrying a plasmid (pLM2) which has an amber mutation in *bla*, the gene coding for this enzyme. The synthesis of β-lactamase in this strain therefore depends on the activity of the suppressing tRNA coded for by the *supF-A81*(Ts) allele.

This system cannot be used to measure the kinetics of turnoff of suppression after a shift from 30 to 42°C because the enzyme synthesized in this strain is itself temperature sensitive. We assume that this is because the insertion of tyrosine or glutamine at the mutated nonsense codon gives rise to a defective enzyme, whereas the wild-type form of the protein has a different amino acid at this site. This can be seen in strain GS101, which was derived by lysogenization of GS100 with λ540 *supF*$^+$. GS101 is resistant to ampicillin (400 μg/ml) at 30°C but sensitive at 42°C.

Figure 1 shows that enzyme activity increased in parallel with cell growth in GS100 at 30°C but then fell rapidly (with half-life of about 20 min)

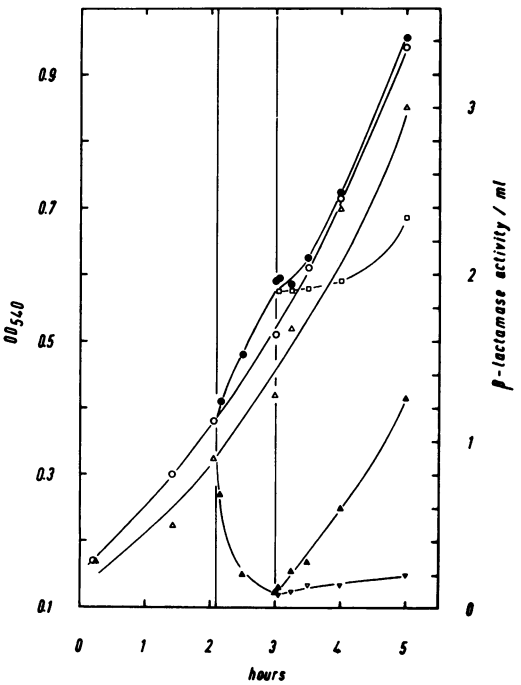

FIG. 1. *Effect of temperature shifts on growth and β-lactamase activity in GS100. A culture was grown at 30°C overnight in NBT, diluted, and grown into mid-log phase. The culture was then divided into two parts (first vertical line), one of which was kept at 30°C while the other was shifted to 42°C (solid symbols). After 55 min at 42°C, this part of the culture was again split into two parts and returned to 30°C with or without chloramphenicol (200 μg/ml). Samples were taken at intervals for β-lactamase assay. Symbols: ○, OD_{540} of 30°C culture; ●, OD_{540} of culture shifted to 42°C and then back to 30°C without chloramphenicol; □, OD_{540} of culture shifted to 42°C and then to 30°C with chloramphenicol; △, enzyme activity (arbitrary units) per ml of 30°C culture; ▲, enzyme activity per ml of culture shifted to 42°C and then 30°C without chloramphenicol; ▽, enzyme activity per ml of culture shifted to 42°C and back to 30°C with chloramphenicol.*

when the culture was shifted to 42°C. After about 1 h at 42°C the culture was divided into two parts and returned to 30°C, either with or without chloramphenicol. The rate of increase in enzyme activity returned immediately to its characteristic 30°C value, and it can be seen that this increase was due to de novo enzyme synthesis and not to reactivation of inactive molecules synthesized at 42°C. The level of suppression therefore returns almost immediately to its characteristic 30°C value when cells which have been growing at 42°C are returned to 30°C.

Kinetics of cell division in asynchronous cultures of OV16. Figure 2 shows cell growth and division in a culture of OV16 [*ftsA16*(Am) *supF-A81*(Ts)] which was shifted during log-phase growth from 30 to 42°C for 60 min and then returned to 30°C. Cell number continued to increase for 10 to 20 min after the shift to 42°C and then remained constant. Cell growth continued during this period, so that median cell volume increased in parallel with culture OD after an initial delay of 10 to 20 min. Identical kinetics of growth and division are seen with the independently isolated strain OV8 [*ftsA8*(Am) *supF-A81*(Ts)] which carries an amber mutation in the *ftsA* gene at a different site from that in OV16. After the return to 30°C, cell growth continued at a reduced rate, but cell division did not resume until after a delay of about 15 min. Cell division thereafter took place more rapidly than cell growth, so that median cell volume returned gradually to its typical 30°C value.

OV16 and OV8 cells growing at 42°C have a characteristic morphology, consisting of long filaments with constrictions at the normal sites of cell division (6). Cells carrying a missense mutation in the *ftsA* gene, resulting in the formation

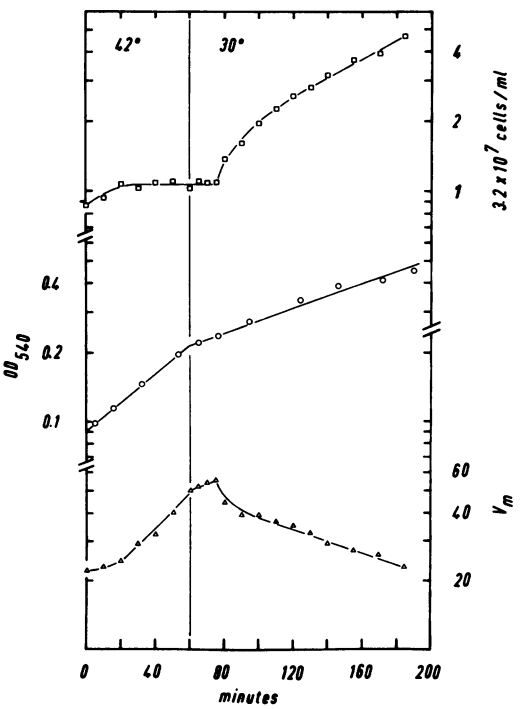

FIG. 2. *Kinetics of growth and cell division in OV16 [ftsA16(Am) supF-A81(Ts)]. A log-phase asynchronous culture growing in NBT at 30°C was shifted to 42°C at 0 min. After 60 min, the culture was returned to 30°C. Symbols: □, cells per milliliter; ○, OD_{540}; △, median cell volume (arbitrary units).*

of a temperature-sensitive protein, also have this appearance after a period of growth at 42°C (13). Figure 3 shows the number and location of nucleoids within an OV16 filament after a period of growth at 42°C. The replication and segregation of nucleoids are evidently normal in this strain. Direct measurement of DNA synthesis (see Methods and Materials) has confirmed that total DNA increases in parallel with OD in OV16 during growth at 42°C (data not shown).

Figure 4 shows cell growth and division at 30 and 42°C in a derivative OV16 which had been lysogenized with the specialized transducing phage λ540 *supF*$^+$. The presence of the temperature-insensitive suppressing tRNA abolished temperature sensitivity for colony formation on plates and also made the cells phenotypically Trp$^+$ at 42°C. It is clear from the figure that the temperature sensitivity for cell division in OV16 is due to the amber mutation.

Synchronous populations. To decide whether *ftsA* protein synthesis is required during the whole cell cycle or during only the last part of it, we have used synchronous cultures (prepared by selecting small cells from asynchronous log-phase cultures by sucrose-gradient centrifugation [8]).

Figure 5 shows the course of division in such a selected population of OV16 cells growing at 30°C. OV16 cells selected in this way do not show as high a synchrony in cell division as do selected cells from the parental OV2 strain. This may be explained by the fact that the distribution of cell volumes in log-phase asynchronous OV16 cultures is much wider than in corresponding cultures of OV2, with median cell volume almost twice that of OV2 but the minimum cell volume approximately equal to that of OV2. Such a distribution could be obtained if the interdivision time in OV16 were more variable than that in OV2. Thus, the time before first division in a selected fraction of small cells would be similar to that for OV2 cells, but successive divisions would be spread over a longer time interval. This explanation would predict that the median (and modal) cell volumes would increase for corresponding times in succeeding cycles, as is seen in Fig. 5. This effect is not seen in synchronous OV2 populations. Figure 5 also shows the effect of adding chloramphenicol at various stages in the synchronous cycle. Cell division stopped about 10 min after the addition of the inhibitor.

Figure 6 shows a similar experiment, except that portions of the culture were shifted to 42°C

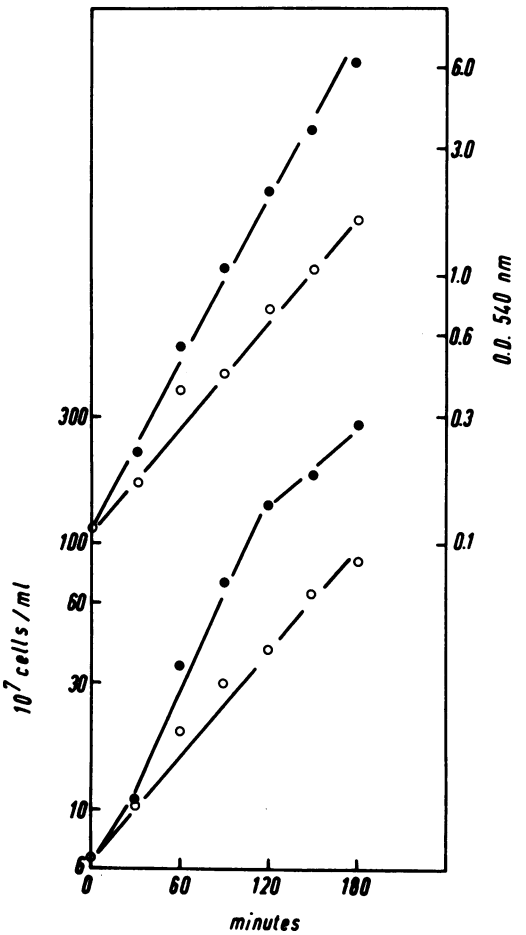

FIG. 4. *Kinetics of growth and division in OV16 lysogenized with λ540 supF*$^+$. *A log-phase culture in NBT at 30°C was divided into two parts at 0 min and growth was allowed to continue either at 30°C (open symbols) or at 42°C (solid symbols). Upper curves show* OD_{540} *and lower curves show cells per milliliter.*

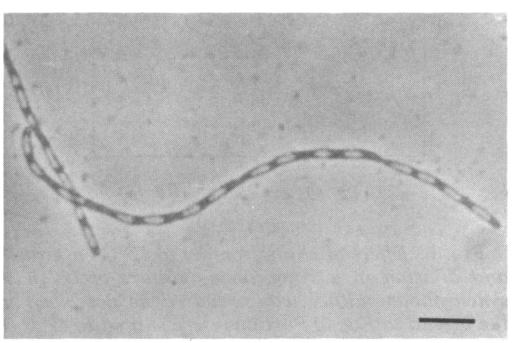

FIG. 3. *Cells of OV16 after 140 min of growth in NBT at 42°C. The cells were photographed under phase-contrast on polyvinylpyrollidone-agar plus chloramphenicol to display the number and location of the nucleoids (light areas). Bar is 5 μm.*

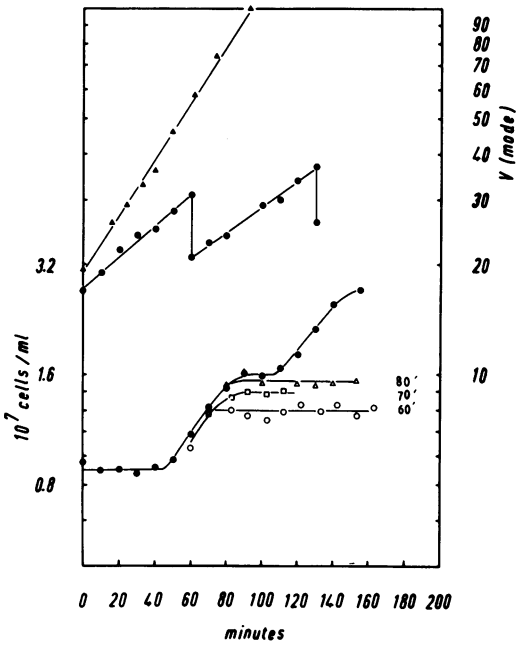

FIG. 5. *Effect of chloramphenicol on cell division in a synchronous culture of OV16 at 30°C. A population of cells from an asynchronous log-phase culture at 30°C in NBT was fractionated by sucrose gradient centrifugation, and a small cell fraction was used to inoculate fresh NBT at 30°C. Portions were taken into chloramphenicol (200 μg/ml) at 60, 70, and 80 min. The lower curves show cell numbers per milliliter. Symbols: ●, untreated control; ○, □, △, chloramphenicol added after 60, 70, and 80 min, respectively. The upper curves show modal cell volume in the untreated 30°C control (●) and in a subculture which was shifted to 42°C at 0 min (▲).*

after various periods of growth at 30°C. As is often seen with this mutant, a small amount of division took place even when the selected cells were shifted to 42°C immediately after gradient selection. However, the division was not increased if the selected cells were allowed to grow at 30°C for up to 40 min before the shift to 42°C. The first cell divisions in the control culture began at some time between 40 and 50 min. If cells were shifted to 42°C after 50 min at 30°C, then cell division began as in the control culture but stopped after 10 to 15 min. The similarity between the kinetics of cell division when chloramphenicol was added to stop all protein synthesis and those seen when only the synthesis of the *ftsA* protein was inhibited suggest that in this strain this protein is one of the last which is required to be synthesized before cell division takes place.

Because suppression in *supF-A81*(Ts) cells resumes immediately after a shift from 42 to 30°C (see above), it is possible to determine whether the synthesis of the *ftsA* protein is required for only a short period before division or whether it must accumulate over a large fraction of the cell cycle. Figure 7 shows the course of cell division in portions of a selected population of small cells growing at 42°C in which portions were shifted to 30°C at intervals. Cells which were shifted after 0 or 15 min began division at about the same time (30 to 35 min in this case, because the selected fraction of cells in this experiment had a somewhat higher median cell volume than those used in the experiments described above; see Fig. 5 to 7). Thus, synthesis of *ftsA* protein is not required earlier than about 15 to 20 min before cell division.

Figure 7 also shows the effect on division of shifting to the permissive temperature after 30, 45, and 60 min at 42°C. In each case cell division appears to resume after a delay of 5 to 10 min, but the amount of division taking place in the first cycle is progressively reduced as the period at 42°C is increased. The amount of cell division seen after cells are shifted to 30°C is approximately equal to the amount of cell division that remains to be completed in the control culture at a time 10 min after the time of the shift. In all subcultures, cell division began once more at

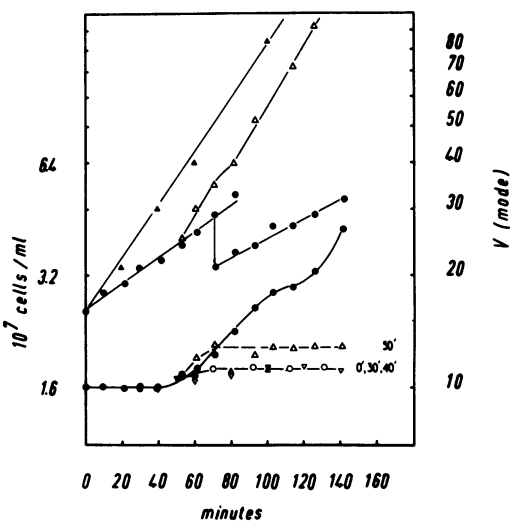

FIG. 6. *Effect of shifts from 30 to 42°C on growth and division in a synchronous culture of OV16. A synchronous culture was prepared as described in the legend to Fig. 5. Portions were shifted to 42°C at intervals. Lower curves show cell numbers per milliliter (●, 30°C control; ▲, ○, ▽, △, shifted to 42°C after 0, 30, 40, and 50 min, respectively). The upper curves show modal cell volume in the 30°C control (●), in the subculture shifted to 42°C at 0 min (▲), and in that shifted after 50 min (△).*

about the same time as the second wave of division in the control culture. The course of subsequent cell division varied from one subculture to the next, but in each case the modal cell volume (and also the median; data not shown) was rapidly reduced to become equal to that of the control culture. Therefore, septa which failed to form because the culture was at 42°C for a part of the first cycle must have been completed in later cycles. It should also be noted that in all of the experiments the time of the first cell division was independent of the size of the cells, which varied in proportion to the periods of time for which the cells had been growing at 42°C.

These experiments indicate that the required time of synthesis of the *ftsA* protein in OV16 cells is a 10- to 15-min period immediately preceding cell division. It would also appear either that cells can make *ftsA* protein only during a short period immediately before division or that the cells are competent to make a septum in response to the synthesis of this protein for only a short period at the end of their cycle.

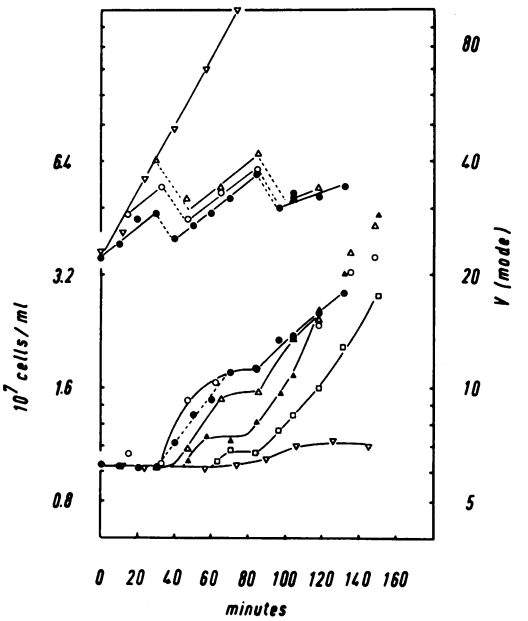

FIG. 7. *Effect of shifts from 42 to 30°C on growth and division in a synchronous culture of OV16. A synchronous culture was prepared as described in Fig. 5, except that the selected fraction of cells was inoculated into NBT at 42°C. At intervals thereafter, portions were shifted down to 30°C. Lower curves show cell numbers per milliliter (▽, culture kept at 42°C throughout; ●, ○, △, ▲, □, subcultures shifted to 30°C at 0, 15, 30, 45, and 60 min, respectively). Upper curves show modal cell volume in the 42°C control (▽) and in the subcultures shifted to 30°C after 0 (●), 15 (○), and 30 (△) min.*

DISCUSSION

Previous work (5, 13) has identified missense mutations at the *ftsA* locus which result in the synthesis of a temperature-sensitive *ftsA* protein. When mutants carrying such a mutation are shifted to 42°C, cell division stops immediately, even if cells are in the process of forming a septum (5, 13). Thus, the *ftsA* protein is required in an active form throughout the entire septation process. The work reported in the present paper suggests that synthesis of *ftsA* protein essential only during a short period immediately before the final stage (cell separation) of cell division. It is not possible to say as yet whether the *ftsA* protein is normally synthesized only at this stage in the cell cycle or whether it is synthesized throughout the cycle but that only those molecules made during the critical period are able to be used for septation. To decide this will require direct measurement of the synthesis of the *ftsA* protein during the cell cycle. Because the *ftsA* protein has now been identified (as a polypeptide with a molecular weight of 50,000 [6]), this approach is feasible. However, it is clear from the present work that accumulation of the *ftsA* protein to some critical amount or concentration during prolonged cell growth is not required but that, instead, a short burst of synthesis at one critical time is sufficient to allow septation to take place.

The actual biochemical activity of the *ftsA* protein is not yet known, but the *ftsA* locus is part of a remarkable cluster of genes, all of which are concerned either with septation or peptidoglycan synthesis, or both (14, 15). Although this group of genes does not form a single operon (6; unpublished data), the fact that all those which are of known function are concerned with enzymes of peptidoglycan metabolism or with penicillin-binding proteins (11) suggests the possibility that all the genes in the cluster have similar functions. The way in which gene expression is controlled within this cluster must make an exciting study for the future.

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