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The *ftsA* Gene Product Participates in Formation of the *Escherichia coli* Septum Structure

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The patterns of septation in filaments of *Escherichia coli*, formed as a consequence of the lack of an active *ftsA* gene product and then returned to permissive conditions, were analyzed in isogenic strains containing three different mutated alleles of *ftsA*. Septation was blocked for at least one doubling time at the potential septation sites that presumably contained inactive FtsA protein but not at those sites containing either the active gene product or no gene product at all. These results suggested a possible structural role for the *ftsA* gene product in the construction of the *E. coli* septum.

The growth of *Escherichia coli* cells may be considered a continuous process that is punctuated by a striking discontinuity called division. The behavior of some mutant strains, such as those containing mutations in a cluster of genes located at min 2.5 of the standard genetic map (1, 5, 7, 10, 12, 14, 16, 17), indicates that some gene products are required for cells to divide. In particular the properties of strains containing either an amber or a missense allele of *ftsA* suggest that the product of this gene is involved in septation (5, 17). Moreover, the fact that the strain containing an amber allele stops dividing in unsuppressed conditions (5) indicates that FtsA activity, as far as cell division is concerned, lasts for a limited time. The molecular events underlying these observations are at present unknown.

As a consequence of the block in septation imposed by *ftsA* mutations, the cells become filaments after a period of growth without division. Filamentation in the absence of FtsA is not lethal, at least during the first stages, and cell division resumes if the filaments are returned to conditions in which active FtsA is produced (17). Within a filament several potential septation sites may be present, and in principle, cell division could take place at any of these when new active FtsA protein is produced. On the other hand, if the choice of division sites among the ones theoretically available in a filament were not at random, it could imply that the *ftsA* gene product may discriminate one site from another provided that the sites within a filament have different properties.

We are reporting here the results observed when septation was resumed in filaments, induced by blocking the activity of the *ftsA* gene product, of three different isogenic strains containing different *ftsA* alleles. These results suggest a possible interpretation of the observed kinetics of FtsA protein utilization (5), because if the FtsA protein is important in the structure of the septum itself, its synthesis should be required for every cell division.

MATERIALS AND METHODS

Strains and growth conditions. *E. coli* strains and their relevant genetic characteristics can be found in Table 1. Phage strains λ16-2 *ftsA*$^+$ and λ16-4 *ftsA*(Ts) were a gift from J. Lutkenhaus (9). Conditions of growth and media have been previously described (11).

Induction of filaments and recovery of septation. Cultures containing 10^7 cells per ml, growing exponentially at 30°C in nutrient broth no. 2 (Oxoid Ltd.), and supplemented with 50 μg of thymine per ml were transferred to 42°C. After a period of incubation at this temperature (45 min for strains D-3 and OV-16 and 60 min for strain D-2), they were transferred back to 30°C. When division resumed (30 min after the transfer to 30°C for strains D-3 and OV-16 and 10 min after for strain D-2), samples were withdrawn and fixed in an equal volume of saline (1.8% NaCl) containing formaldehyde (0.8%). Throughout the experiments, samples were also taken for optical density and particle number measurements. (These optical density and particle controls did conform to the expectations and are not shown.)

Cell parameter measurements. Particle numbers, measured with a Coulter Counter, and optical density measurements at 450 nm were done by previously published procedures (11). Cell length and septum-to-pole distances were measured in magnified prints from negatives obtained with a Zeiss photomicroscope III, by using the fixing and photographic procedures described by Donachie et al. (6). The measurement of distances involved the measurement of meandering filaments and was done with the help of a Hewlett Packard 9111A graphics tablet coupled to a HP-85 computer. The software, program MLENG, was based on the 9111A-HP-85 system tutorial that includes a continuous digitizing option (details can be supplied on request). Graphic representation of data was done with the program DPLOT supplied by Hewlett Packard in the HP-85 Standard Pac, and printouts from the computer were redrawn for reproduction.

Genetic procedures. Procedures for transduction with P1 and complementation with λ vectors have been published previously (11).

RESULTS

Identification of the *ftsA2* allele. Strain MAC2, which contains a temperature-sensitive mutation (J. E. Llamas, unpublished data), was made *leu*$^+$ by P1 transduction from MB188. The ts mutation was then cotransduced with *leu*$^+$ into OV-2 (cotransduction frequency, 40%). The resultant strain, D-2, was lysogenized with λ16-2 *ftsA*$^+$, and the lysogens were temperature resistant. Displacement of the prophage by λ *b2red* (11) led to the recovery of the mutant phenotype. Lysogenization with λ16-4 *ftsA*(Ts) failed to complement the mutation present in D-2. The morphology of strain D-2 dislocated filaments at 42°C was in agreement with the complementation data identifying the mutation present in D-2 as an allele of *ftsA* (17).

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TABLE 1. *E. coli* K-12 strains used in this work

Strain	Genetic markers	Source or reference
MB188	F⁻ *trp*(Am) *ara*(Am) *lac125*(Am) *galKu42*(Am) *galE tyrT* (*supFA81*)(Ts)	L. Errington
OV-2	As MB188 *leu ilv his thyA deo tsx*(Am)	6
MAC2	F⁻ *thi thr leu proC thyA deoB deoC ftsA2*(Ts)	J. E. Llamas
D-2	As OV-2 *leu*⁺ *ftsA2*(Ts)	This work
D-3	As OV-2 *leu*⁺ *ftsA3*(Ts)	17
OV-16	As OV-2 *ftsA*(Am)	5

This temperature-sensitive allele of *ftsA* differed from our previously described *ftsA3* allele (17) in one important property: division of temperature-induced filaments of D-2 (more than a 60% increase in particles) can occur when it is returned to the permissive temperature even when new protein synthesis was prevented by chloramphenicol (Fig. 1). This result indicated that, although the FtsA protein made by strain D-2 at 42°C is not active, it may become active at 30°C. Otherwise the behavior of D-2 was similar to other *ftsA* alleles (A. Tormo, unpublished data). We propose the designation of *ftsA2* for this allele.

Localization of septa within dividing filaments. Figure 2 shows two different representations of the theoretical positions of potential division sites within a filament, relative to the length of the filament. It was assumed that cell length can be measured in units and that a cell of 2 units had one potential division site in the middle. As it continued growing, new potential septation sites appeared, one additional site being added per each unit of length, with the requirement that the minimum possible distance from one site to the nearer one, or to the nearer pole, be one unit (see also reference 4).

The graphic representations in Fig. 2 are equivalent and are modifications of the graphic forms used by Donachie and Begg (4). As the two poles of a filament are, for our purposes, formally equivalent, the position of each septum has an axis of rotational symmetry, perpendicular to the cell length, in the middle of the filament. In Fig. 2B, the position of each septum relative to the nearest pole should then be represented in duplicate, even for those septa that occupy exactly the central position. This was achieved by representing the distance from each septum to each pole. Within one filament, potential division sites of different ages were present, depending on the length of the filament (see Fig. 2).

Septum location in filaments formed as a consequence of mutations in *ftsA*. Although the absence (5) or inactivation (17) of *ftsA* inhibits septation, the cell may be able to resume division upon a shift back to permissive conditions (see Fig. 3 through 5). Viability of D-3, as measured by the number of colonies formed on plates at 30°C, does not decrease at 42°C during incubations lasting 60 min (17). Filaments formed during incubation at 42°C should therefore be able to divide after a period of growth at 30°C. However, D-2 differed slightly from D-3 and OV-16; D-2 started dividing 10 min after the shift back to 30°C, whereas 25 min were required by the other strains. For the next experiment we then chose a total incubation period at 42°C plus recovery at 30°C equal to 2.5-length-doubling times of each strain under these experimental conditions. This timing ensured that filaments of equivalent length, and therefore containing septa of equivalent ages, were measured in the three strains. The pattern of septation found during recovery at 30°C depends on the particular *ftsA* allele harbored by each strain (Fig. 6).

The potential septation sites of D-3 that most probably had been formed at the restrictive temperature, namely those of age 0 in filaments of up to 4 units and those of age 1 in filaments of up to 8 units, seemed to be preferentially blocked. Septa of age 0 in 8-unit filaments were probably formed before the temperature shock but were blocked by inactivation of FtsA before being completed. We reasoned that, if the block was a consequence of the impaired *ftsA* gene product, the pattern of septation of strain D-2 during recovery should show no preferential inactivation of any position because the *ftsA* product in this strain should recover its activity upon the shift back to 30°C. Other conditions being the same, no preferential inactivation of any potential septation site should occur in OV-16 either, because this strain should synthesize a negligible amount of FtsA protein in unsuppressed conditions. The pattern of septation obtained for D-2 and OV-16 (Fig. 6) was compatible with a mode of action of FtsA in which the active protein works at any of the available potential division sites with the same probability, although in all the cases a higher percentage of division at the positions near the poles was observed.

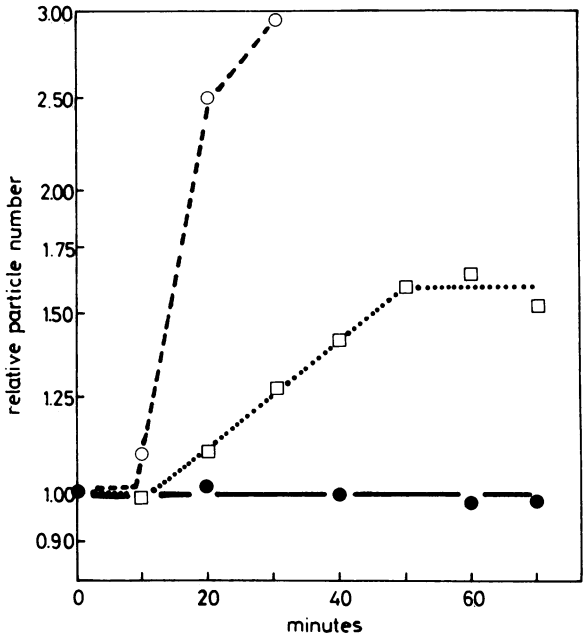

FIG. 1. Recovery of division in strain D-2, in the presence and absence of protein synthesis, after division inhibition caused by a thermal shock. An exponentially growing culture of D-2 was shifted from 30 to 42°C and kept at this temperature during 60 min. At time zero, one portion was returned to 30°C (○), a second portion was returned to 30°C and chloramphenicol (200-μg/ml final concentration) was added simultaneously (□), and a third portion was kept at 42°C (●) without any additions. At the times indicated in the graph, samples were withdrawn and particle concentration was measured as described in the text. Particle concentration at time zero was 2.42×10^7 particles per ml.

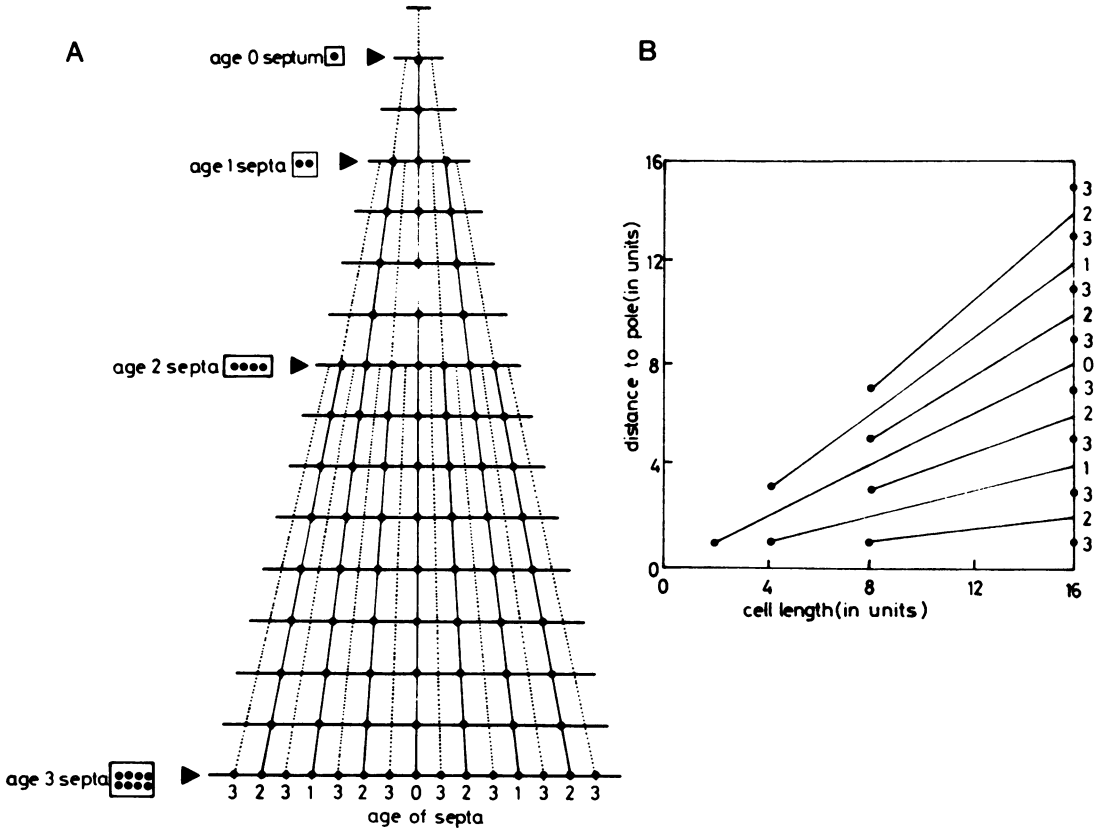

FIG. 2. Graphic representations of the theoretical position of septa within filaments of different lengths. (A) The elongation of a cell measuring one theoretical unit of length. Length in the horizontal scale increases linearly, whereas the vertical age scale is exponential. One, two, four, and eight new potential septation sites are added at ages 0, 1, 2, and 3, respectively. The age of potential septation sites present within a filament measuring 16 length units is indicated at the bottom. Thin, continuous lines keep track of individual septa. Dotted lines mark the places in which new septation sites will be added. (B) A formally equivalent representation (modified from reference 4) in which the time scale has been substituted by a cell length scale in the abscissa on the assumption that elongation is a function of time. The length scale is modified to represent distances from a potential septation site to each of the poles of a filament and is represented now in the ordinate. Both scales are linear in this form used to plot the data shown in Fig. 6. Ages of sites are now indicated at the right. The lines in (B) are equivalent to the thin, continuous lines depicted in (A).

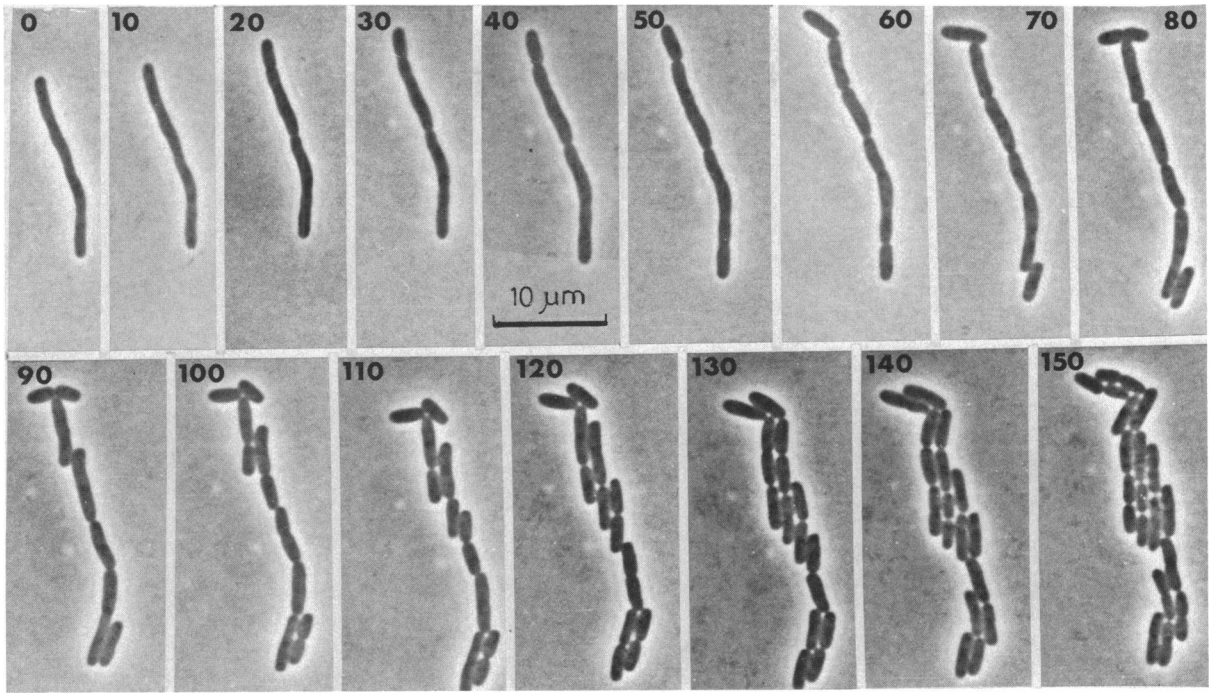

FIG. 3. Recovery of septation at 30°C in filaments of strain D-2. A culture of D-2 growing at 30°C in nutrient broth containing thymine (50 μg/ml) was shifted to 42°C to induce filamentation during 1 h. At time zero a loopful of the culture was placed on top of a slide coated with nutrient agar containing thymine and observed at room temperature (20°C). Photographs were taken by using phase-contrast optics, every 10 min.

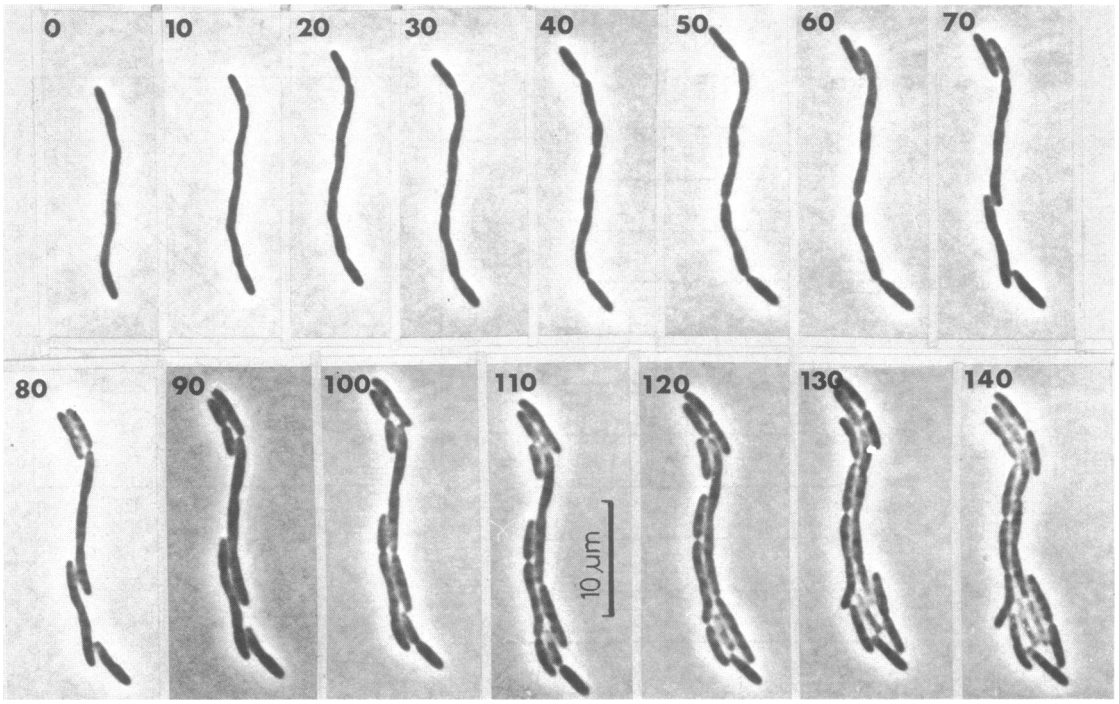

FIG. 4. Recovery of septation at 30°C in filaments of strain D-3. Experimental procedure is described in the legend to Fig. 3.

The block of the potential septation sites in strain D-3 does not seem to be totally irreversible because in filaments of up to 8 units several divisions occurred in the middle of the filament at a position of age 0. We could not test the prediction that the block lasts for only one doubling time by observing the recovery of septa of age 1 in D-3 filaments of up to 16 units, because these filaments cannot be obtained under the conditions of our experiment. Modification of our experimental design would be impractical because lengthening the incubation time at 42°C would lead to decreased viability (17) and increasing the recovery time at 30°C would cause cell separation.

DISCUSSION

In this work we addressed the question of whether the active *ftsA* gene product of *E. coli* does reach all the

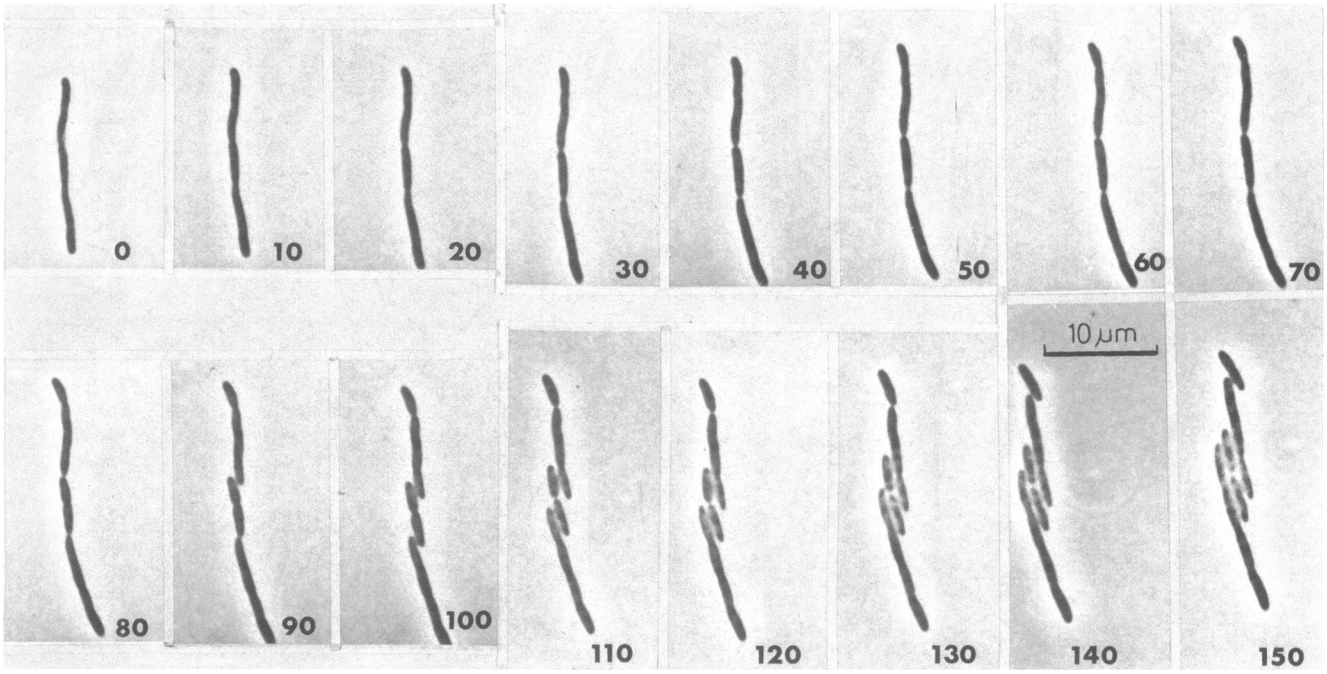

FIG. 5. Recovery of septation at 30°C in filament of strain OV-16. Experimental procedure is described in the legend to Fig. 3.

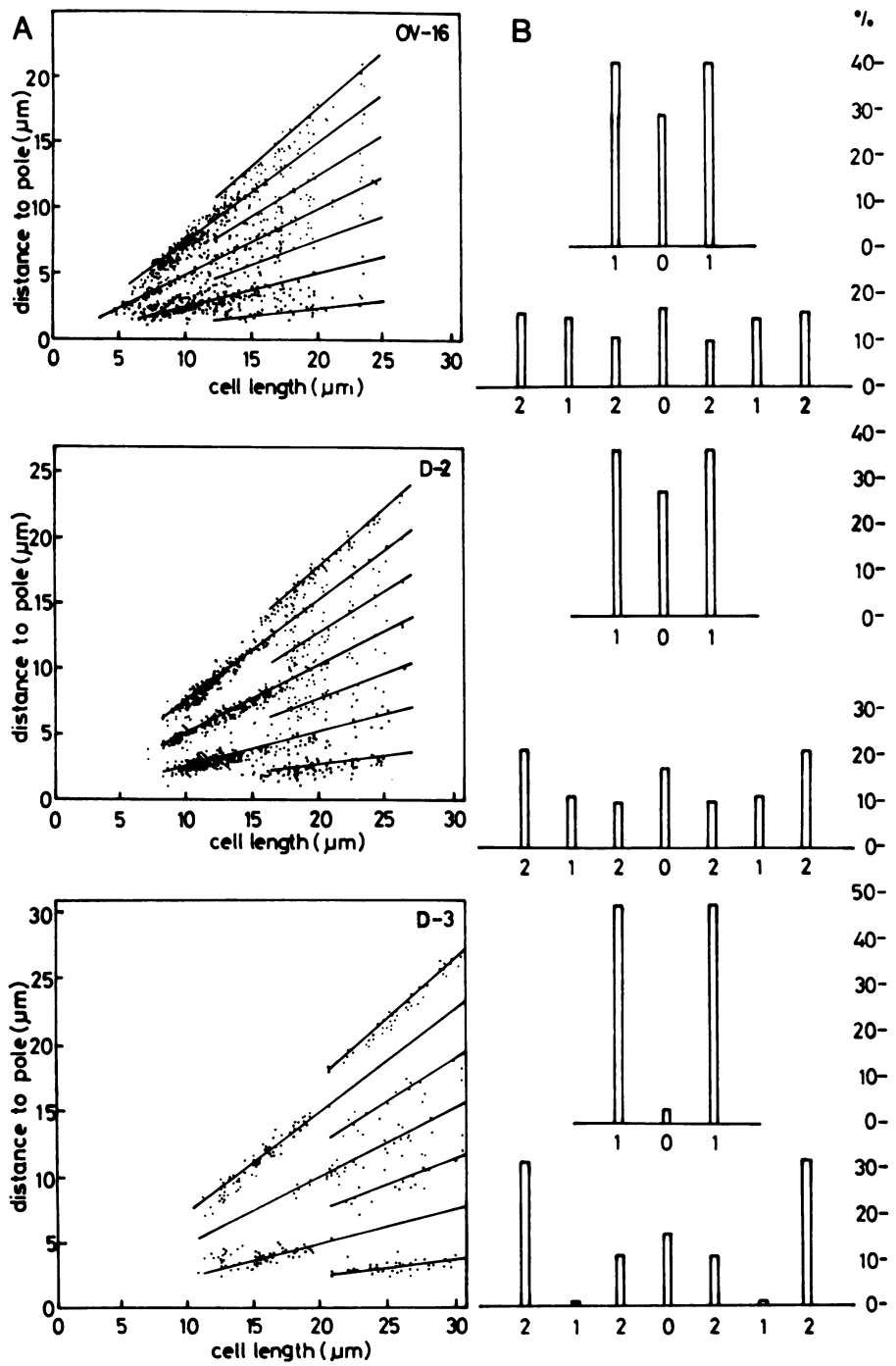

FIG. 6. Localization of septa in filaments formed in the absence of active *ftsA* gene product. (A) Length of each filament and the distance of each septum to each pole. The lines inside the panels are not regression lines but the theoretical lines calculated in a fashion similar to that in Fig. 2. To calculate the histograms plotted in (B), the data of 4-length-unit-sized filaments were pooled in one set (top of each pair) and those of 8 units in another (bottom). Filaments were assigned to one or the other set according to their length; the border of the two sets was considered to be that length in which the potential septation sites increased from three to six (estimated from the graph). Septa were then assigned to each potential site bisecting the surface between each pair of theoretical lines. The vertical scale indicates the percentage of total septa appearing at each position. The ages of septa are indicated by the same convention used in Fig. 2. Septa of age 0 in 4-unit filaments were those formed during incubation at 42°C; septa of age 1 in these filaments were formed during recovery at 30°C. Similarly, septa of age 0 in 8-unit filaments were either initiated at 30°C and inactivated at 42°C or formed early during incubation at 42°C; septa of age 1 were formed during incubation at 42°C; and septa of age 3 were mostly formed during recovery at 30°C, although the possibility that some septa of age 3 had their origin in the latter part of the incubation at 42°C cannot be totally excluded. The total number of septa and the total number of filaments measured (septa/filament) were 734/442 in strain OV-16, 675/367 in strain D-2, and 193/142 in strain D-3.

available potential division sites with an equal probability. Results for strain OV-16 (Fig. 6), in which under unsuppressed conditions the synthesis of FtsA decreases to less than 1% (2), indicated that the probability of septation at a given place after recovery of FtsA synthesis to a level of 13% was almost equal for all the available sites.

In strain D-2, in which the inactivation of FtsA at 42°C can be reversed at 30°C, filamentation did not inactivate the potential septa that were able to resume septation upon a shift back to permissive conditions. On the other hand, only those septa of strain D-3 that were formed at 42°C were not able to resume division, whereas those formed during the recovery at 30°C were able to do so. We interpret this result as being a consequence of a block established in the septa by the *ftsA3* gene product that had been irreversibly inactivated at 42°C. In accordance with this interpretation, the potential septa in strain OV-16, in which no significant amount of FtsA protein was made at 42°C, were not preferentially inactivated at any position during filamentation at the restrictive temperature. The simplest alternative to explain these results would be that the FtsA protein is incorporated into the structure of the septum. Other alternatives, such as that it may act on some component of the septum without actually forming part of the structure, cannot be totally excluded at this stage by our mainly genetic evidence.

Real septa are likely to be complex structures that interact with the rest of the cell. Under normal circumstances, bacterial cells are relatively simple and contain only one potential septation site per cell, but our results were obtained in filaments in which local differences would be enhanced by distance. It is therefore possible that the elevated septation found at the positions adjacent to the poles (for example in 4-unit filaments of strains OV-16 and D-2 in Fig. 6) could be due to effects present only in filaments.

Mean cell length, and therefore length for a particular cell age, in *E. coli* is a constant for a given growth rate (6, 13). This constancy could be the result of passive processes, such as the periodic attainment of the initiation mass for DNA replication (3), that would impose a timing on cell division independent, in principle, of cell length. Another alternative would be the operation of a positional mechanism that could place the septum at a certain length according to the growth rate. When cell division is inhibited by the expression of an *ftsA* mutation, no effect on DNA replication or mass accumulation (measured as optical density increases) has been observed (5, 17; Tormo, unpublished data). Our observations show that upon return of the *ftsA* mutants to conditions in which FtsA is active, division did not occur randomly along the total length of the filaments; septa only appeared at preferred positions separated from each other by at least a minimum distance (Fig. 6). Moreover, the spacing of septa was not solely the result of passive timing exerted by the initiation mass, because septa appeared in recovering filaments with an abundance dependent on the *ftsA* allele (i.e., septa were present at almost every possible position in D-2 filaments but only at a few positions in D-3 or OV-16 filaments) (see Fig. 3 through 5 and the legend to Fig. 6). If initiation mass (3) or chromosome termination (8) were then to impose a timing on septation, they could do so by triggering the assembly of some primordial septum structure in the cell envelope that could include the *ftsA* gene product.

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