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Amber Mutation Affecting the Length of *Escherichia coli* Cells

ENCARNACIÓN MARTÍNEZ-SALAS AND MIGUEL VICENTE*

Instituto de Biología Celular, Consejo Superior de Investigaciones Científicas, Velázquez 144, Madrid-6, Spain

An amber mutation in a newly found gene (*wee*) of *Escherichia coli* has been isolated from strain OV-2, which harbors a temperature-sensitive suppressor. At 42°C cells of the mutant, OV-25, increased in mass and deoxyribonucleic acid content and divided at normal rates, compared with the wild type under the same growth conditions. Total cell length increased under the restrictive conditions, although at a slightly lower rate. Values of mean cell length and cell volume, contrary to what would be expected from the increment in the rate of increase in particles, mass, and deoxyribonucleic acid, became at 42°C smaller than those found in the wild type. A parallel increase in protein content per length and cell density and a loss of viability were found to occur after four generations at the restrictive temperature. The behavior of strain OV-25 in the absence of the *wee* gene product could be interpreted in terms of either a faulty regulation of the elongation processes or their abnormal coordination with the cell cycle. The genetic location of the *wee* gene has been found to be at 83.5 min on the *E. coli* genetic map.

Mean cell length in *Escherichia coli* has been described as being dependent on the growth rate of the culture: cells are longer at fast growth rates and shorter at slow ones (6). Cell elongation during the cell cycle follows a linear course, with a doubling in rate at a given time before division (4, 6), although some reports describing exponential elongation have been published (11). Cell elongation does not seem then to be a simple process, as it is responsive both to cell age and to changes in the overall growth rate of the cell. On the other hand, to maintain at a given growth rate the mean cell length and length at birth within the narrow limits that are observed (4, 6), some sort of coordination should exist between elongation and division.

At least one of the inner membrane proteins that bind penicillin covalently plays a role in cell elongation (18). One mutant in which this penicillin-binding protein, PBP 1Bs, is impaired has been described, and its genetic location within the *E. coli* genome was found at 3.3 min on the standard genetic map (19). This alteration of PBP 1Bs stops elongation and leads to cell lysis at the restrictive temperature.

The complexity of the cell elongation processes, together with the need to coordinate elongation and division, makes it reasonable to believe that the products of more genes, in addition to PBP 1Bs, should be directly involved in elongation, not only at a synthetic level but also in its regulation and coordination with other cell cycle events. We describe here an *E. coli* strain in which cell length is affected as a consequence of a nonsense amber mutation at a different locus on the *E. coli* map. This strain is a derivative of a K-12 strain, OV-2, for which a study of cell length was previously published (6).

MATERIALS AND METHODS

Bacterial strains and growth conditions. Table 1 lists the strains used in this work and their relevant genetic characteristics. Cultures of OV-25 and its derivatives were grown in gyratory water bath shakers at 30 or 42°C. Oxoid nutrient broth no. 2 containing 50 μg of thymine per ml was used, except for DNA replication measurements, for which 20 μg/ml was used. M9 salts (3) medium with various supplements and the requirements of the strain at 20 μg/ml (except thymine, which was used at 50 μg/ml) was used as described below in several experiments. Cell concentration was kept lower than 3×10^7/ml by dilution with prewarmed medium. Growth (optical density and particle increases) was followed during at least two doublings and found to be parallel before the start of each experiment.

Phage strains. P1 *kc* was used for transductions, λ540 (*tyrT*) (14) was used to produce suppressed lysogens, λ *b2 red* (10) was used to cure the lysogens, and λ *vir* was used to distinguish lysogens from λ-resistant strains.

Mutagenesis and selection. Nitrosoguanidine at a final concentration of 100 μg/ml in 0.1 M Tris-maleate, pH 5.5, was used as a mutagen. After mutagenesis of strain OV-2, which carries a temperature-sensitive suppressor (see Table 1), temperature-sensi-

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

Strain	Genotype/phenotype	Source or derivation
KL 25	Hfr PO46, *supE44*	CGSC[a] 4244
KL 209	Hfr PO18, *malB16* λ^r *supE44*	CGSC 4315
M6K	F$^-$ *leu thyA* (*deo*) *ara*(Am) *lac-125*(Am) *galU42*(Am) *gal* epimerase *trp*(Am) *tsx*(Am) *tyrT* [*supF*-A81(Ts)]	Reference 6
M6K *bgl*	As M6K, *bgl*	This work
OV-2	As M6K, *ilv, his*	Reference 6
OV-25	As OV-2, *met*(Ts) *arg*(Ts) *wee*(Am)	This work
OV-25.1	As OV-25, *nal*	This work
OV-25.2	As OV-25, λ540 (*tyrT*) lysogen	This work
OV-25.3	As OV-25, *tyrT*	This work
OV-25.4	As OV-25, *wee*$^+$ (revertant)	This work

[a] Coli Genetic Stock Center.

tive (42°C) clones (1% of the survivors) were isolated by replica plating onto nutrient agar plates. OV-25 is one of the temperature-sensitive clones that were classified as amber mutations, rather than missense, based on the ability of λ540 (*tyrT*) lysogens to grow at the restrictive temperature (see Results; M. Vicente and N. Otsuji, unpublished data).

Revertants were obtained spontaneously and were classified as reverted either in the temperature-sensitive suppressor or in the mutated gene according to their ability to grow or not at 42°C in the absence of tryptophan (because OV-2 carries a *trp* amber mutation; Table 1).

Nalidixic acid-resistant derivatives were obtained as spontaneous mutations after cells from an overnight liquid culture, to which 20 μg of nalidixic acid per ml was added at early exponential phase, were plated onto nutrient agar plates containing 20 μg of nalidixic acid per ml.

The *bgl* derivatives were spontaneous mutations selected on minimal (M9) agar containing 0.2% salicin as a carbon source in addition to the requirements of the strain.

Cell parameter measurements. Optical density at 540 nm was measured in a Gilford 300 N microsampler spectrophotometer.

Cell length was measured from enlarged negatives obtained by use of a Zeiss Photomicroscope III as described by Donachie et al. (6).

Particle counts were made with a Coulter Counter ZBI (30-μm orifice; amplification, 2; aperture current, 1; lower threshold, 10; upper threshold, off).

Volume measurements were obtained from the Coulter Counter by means of a Coulter Channalyzer (base channel threshold, 5; window width, 100) and an X-Y plotter.

DNA synthesis was followed by incorporation of [^{3}H]thymine (100 μCi/ml) into 10% trichloroacetic acid-precipitable material. Cultures were grown in the labeling mixture at 30°C for at least four generations before the start of the experiment.

For measurements of the cell protein content, 25-ml samples containing around 10^8 cells per ml were cooled and fixed with formaldehyde at a final concentration of 0.09% (vol/vol) and 2.16 mg of NaCl per ml. The cells were pelleted by centrifugation and washed with 10 ml of saline (8 mg of NaCl per ml and 0.8 mg of sodium azide per ml). They were pelleted again and suspended in 1 ml of distilled water, and their proteins were denatured by precipitation with 10 ml of chilled 10% trichloroacetic acid. The precipitate was pelleted by centrifugation, dissolved in 1 ml of 0.1 M NaOH, and heated for 5 min in a boiling-water bath. Proteins were measured in an adequate dilution of this extract by the method of Lowry et al. (12).

Cell density was measured in 16% Ludox HS40 (Du Pont Co.) gradients by the procedure described by Poole (17) except that no polyvinylpyrrolidone was added, thus producing a much steeper gradient upon centrifugation to equilibrium at $6 \times 10^4 \times g$ (21). After centrifugation, fractions containing 20 drops (around 0.8 ml) were syphoned, starting at the level just above the solidified silica pelleted at the bottom of the tube. Particle concentration in each fraction was determined with a Petroff-Hausser chamber (Ludox micelles produced a very high interference in the Coulter Counter). The density of the fraction was calculated from its refractive index by interpolation in a calibration curve obtained from measurements of the refractive index of Ludox solutions of known densities ranging between 1.0 and 1.10 g/ml.

Genetic procedures. Matings were interrupted at different times by dilution into M9 salts containing 100 μg of nalidixic acid per ml and plating on selective plates containing 20 μg of nalidixic acid per ml as described by Zipkas and Riley (23). The donor to receptor ratio was 1:10.

Transductions were performed as described by Masters (13).

RESULTS

Isolation. Temperature-sensitive derivatives of strain OV-2 can arise through either of two different types of mutation. They can be either temperature-sensitive missense mutants or amber mutants suppressed at the permissive temperature of *supF*(Ts) A81 (30°C). Strain OV-25 was isolated after nitrosoguanidine mutagenesis as a strain unable to plate on nutrient agar at 42°C and was found to belong to the class of amber mutants by its ability to grow at the restrictive temperature for *supF*-A81(Ts) (42°C) when lysogenized wtih phage λ540 (*tyrT*), which carries a temperature-resistant suppressor. Furthermore OV-25 λ540 (*tyrT*) lysogens could be cured of the prophage by displacing it with λ *b2 red*; of the three clones so cured, all were again temperature sensitive, thus corroborating the nonsense nature of the mutation.

General characterization. At the restrictive temperature strain OV-25 behaved as an auxotroph, probably due to temperature-sensitive secondary mutations resulting from nitrosoguanidine mutagenesis (8). That these temperature-sensitive requirements were methionine and arginine was found in a strain, OV-25.4, that was a revertant able to plate on nutrient agar at 42°C

(see Table 1). Strains lysogenic for λ540 (*tyrT*) were also found to behave as temperature-sensitive auxotrophs, which indicates that the auxotrophic mutations are not amber. At the permissive temperature, cells of OV-25 increased in number at rates comparable to the wild type (Fig. 1 and reference 6). At the restrictive temperature in rich medium, the growth rate was the same as that of the wild type for at least six doublings; after this, the cells eventually stopped growing and there was no further increase in particle numbers. No lysis was observed during the experiment. At the permissive temperature cells were slightly longer than the wild-type cells (see below) and less than a 5% of them were filamentous (longer than 6.58 μm). When shifting to the restrictive temperature, the proportion of filaments relative to the main population decreased, although they continued elongating, as could be judged from their frequency in length and volume distributions. They have therefore been excluded from the calculations of mean cell length at both temperatures (except when indicated in one experiment).

Cell length in the absence of the *wee* gene product. Cells of OV-25 grew in nutrient broth at 30°C with doubling times between 45 and 55 min. Mean cell length at this temperature was slightly larger than that of the parental strain (OV-2; reference 6). When shifted to 42°C, the expected acceleration in the rates of particle increase, optical density increase, and DNA synthesis was found with doubling times between 25 and 30 min (Fig. 1). The rate of total cell length increase accelerated too, although its doubling time was slightly longer (35 min). A deceleration in the rates of increase in cell counts and total cell length was found after five doublings. The course followed by total cell length could be understood by considering the decrease in mean length found for OV-25 populations at 42°C shown in Fig. 2.

From the results of Donachie et al. (6), the mean cell length for a growth rate such as the one found under these conditions can be calculated. Their simplified equation

$$\bar{L} = (2 + 2R/3)\ \mu m \qquad (1)$$

(where $R = 60/\tau$, τ being the generation time) gives a value of $\bar{L} = 3.60\ \mu m$ for $\tau = 25$ min. For wild-type cells it would be expected that mean cell length would increase after changing from $\tau = 45$ min to $\tau = 25$ min. Contrary to these expectations, as can be seen in Fig. 2 and 3, mean cell length of the mutant strain OV-25 was shorter when it was growing at 42°C at faster rates than when it was growing at 30°C at slower ones. Mean cell length after 240 min at 42°C in OV-25 remained constant at a value of 2.47 μm (standard deviation, 0.15) at growth rates ranging from 0.67 to 2.4 doublings per hour (Fig. 3). At 30°C mean cell length, although larger than that observed in the wild type (OV-2), was nevertheless sensitive to the changes in growth rate,

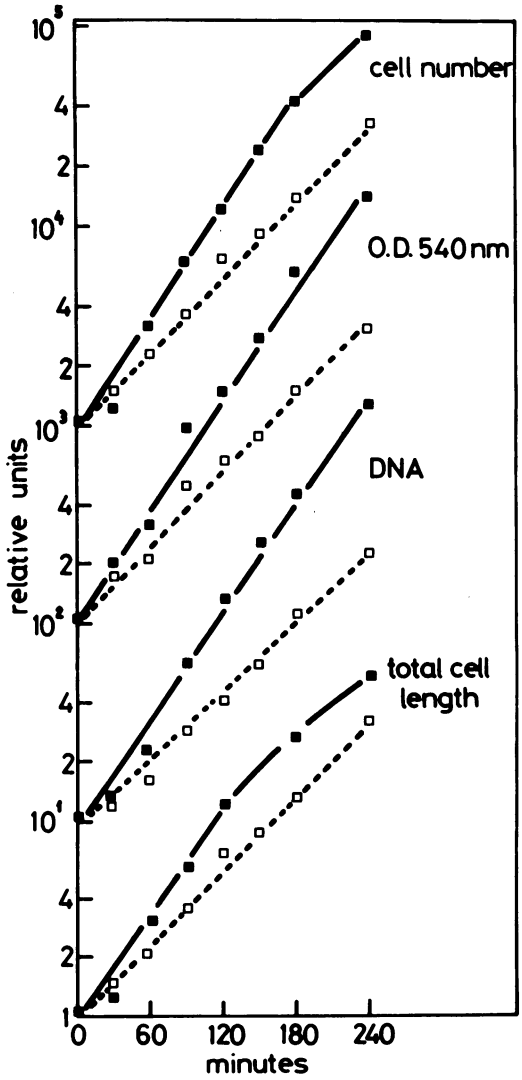

FIG. 1. *Growth of OV-25 at 30 and 42°C. A culture of OV-25 growing exponentially at 30°C in nutrient broth plus thymine was split in two at time zero. One part was kept at 30°C (open symbols and broken lines); the other part was shifted to 42°C at time zero (filled symbols and solid lines). Samples of each culture were withdrawn at intervals, and cell parameters were measured as indicated in the text. Total cell length was calculated as: mean cell length × cell number/cell number at zero time. Initial values were 4.9 × 10⁷ cells per ml, 0.220 absorption units, 1.3 × 10³ cpm/ml, and 4.08 μm.*

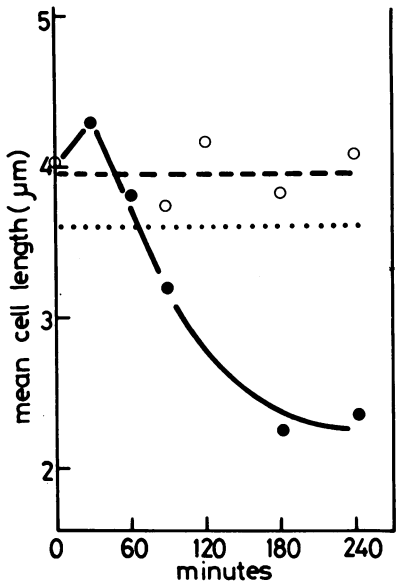

FIG. 2. *Mean cell length of OV-25 at 30 and 42°C. Mean cell length was measured in the samples obtained in Fig. 1. For length measurements, no fewer than 200 cells were measured for each point. The dotted line is the value calculated from equation 1 (see text) for a generation time of 25 min. Other symbols as in Fig. 1.*

increasing as the generation time became shorter.

Using the nomenclature of Nurse et al. (15) for *Schizosaccharomyces pombe*, we propose to call the gene responsible for this phenotype in OV-25 *wee*, as the cells become shorter when the mutation is expressed at 42°C.

Cell volume in the absence and presence of the *wee* gene product. A simple microscopic examination revealed no gross changes in the diameter of OV-25 cells when shifted to 42°C, compared with the wild type. Results in Fig. 4a showed that the decrease in length at 42°C was accompanied by a decrease in cell volume relative to the volume of the *wee* strain growing at 30°C. As volume measurements in the Channelyzer are a much more rapid method than cell length or diameter measurements, this was the method used to find whether lysogens of λ540 (*tyrT*) that had recovered their viability at 42°C had a normal volume, relative to the wild type, at this temperature. Figure 4b shows that this was the case; the recovery of viability was accompanied by values of volume that were slightly greater than those found for the wild type, the volume at 42°C being larger than at 30°C, as would be expected from having a faster growth rate.

Cell volume at 42°C was found to increase normally (relative to the volume at 30°C) in *wee*$^+$ revertants as well as in temperature-resistant suppressor revertants of *SupF*-A81(Ts) (data not shown). This corroborated the nonsense nature of the mutation in OV-25. A small percentage of filamentous cells was nevertheless found to be present in all these cultures, even in suppressed and reverted strains.

Changes in mass per cell in the absence of the *wee* gene product. If OV-25 cells grow at the restrictive temperature at rates comparable to those found in the wild type in the same medium (6), but mean cell length and volume are much smaller than those of OV-2, it then follows that both mass per unit of cell length and per unit of volume should increase during the course of a temperature shift. Measurements of optical density per cell confirmed this expectation (data not shown). To find whether the observed increase in optical density per cell was correlated to an increase in cell mass, we performed experiments in which the amount of protein per cell was measured at 30°C and after a shift to 42°C, both in OV-25 and in the wild

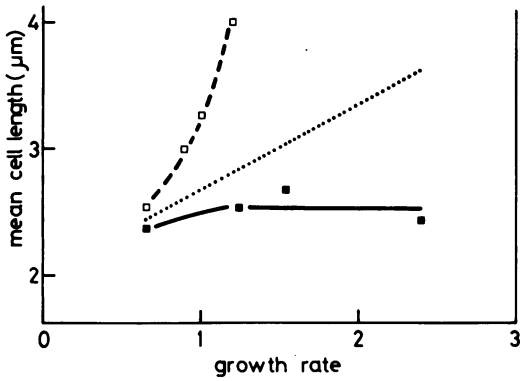

FIG. 3. *Dependency of mean cell length of OV-25 on growth rate. Mean cell length, calculated as in Fig. 2, was measured at 30°C (open symbols, broken line) and 240 min after a shift to 42°C (filled symbols, solid line). Media to obtain different growth rates were nutrient broth plus thymine (50 μg/ml) (45 min at 30°C; 25 min at 42°C); M9 plus glucose (4 mg/ml), cytosine (20 μg/ml), adenine (20 μg/ml), uracil (20 μg/ml), Casamino Acids (5 mg/ml), OV-2 requirements (20 μg/ml), and thymine (70 min at 30°C; 40 min at 42°C); M9 plus glucose, Casamino Acids, OV-2 requirements, and thymine (62 min at 30°C; 50 min at 42°C); M9 plus glycerol (8 mg/ml), OV-2 requirements, methionine (20 μg/ml), arginine (20 μg/ml), and thymine (90 min at both temperatures). The dotted line serves as reference and is derived for OV-2 from equation 1 and reference 6 for growth rates falling within the range observed for OV-25 in this figure.*

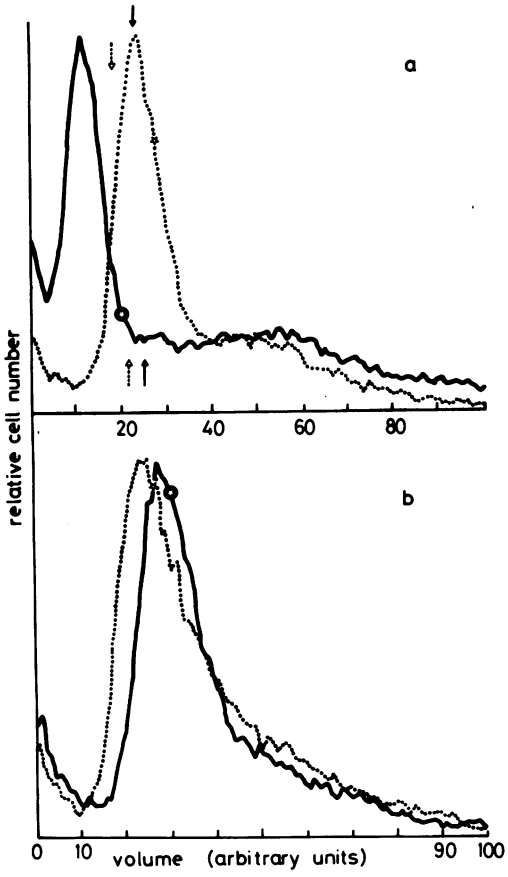

FIG. 4. *Cell volume distributions in the absence or presence of the wee gene product. Dotted lines, 30°C; solid lines, 42°C. (a) Volume distributions of OV-25 180 min after being shifted to 42°C. (b) Volume distributions of OV-25.2, a λ540 (tyrT) lysogen of OV-25, under the same conditions. Graphs are redrawn from the output of an X-Y plotter connected to the Channalyzer. On each graph the median of the distribution is indicated by a star. Arrows pointing upwards mark the medians at 30°C (broken line) and at 42°C (solid line) of OV-2. Arrows pointing downwards mark the modes for OV-2 at 30°C (broken line) and 42°C (solid line). Medium was nutrient broth plus thymine.*

type. Results from one such experiment are shown in Fig. 5a. At 30°C the amount of cell protein was greater for OV-25 than for the wild type, as could be predicted from its longer mean length and larger volume. A shift to 42°C resulted in an increased amount of protein per cell in the wild type, as both length and diameter were expected to increase after the shift (6, 16). If a shift to the restrictive temperature would impair mass growth in OV-25, it would be expected that the amount of protein per cell at 42°C would either remain at the same value found at 30°C or diminish. This is not what was found; on the contrary, the amount of protein per cell, under restrictive conditions, reached values twice as high as those found in the wild type. What is more, no steady state was reached in the mutant, whereas the wild type reached the steady state two generation times after the shift to the higher temperature.

These results could be criticized because the amount of filaments present in OV-25 populations, although less than 5%, could play a substantial role in increasing the average cell mass. That this was not true is shown in Fig. 5b, in

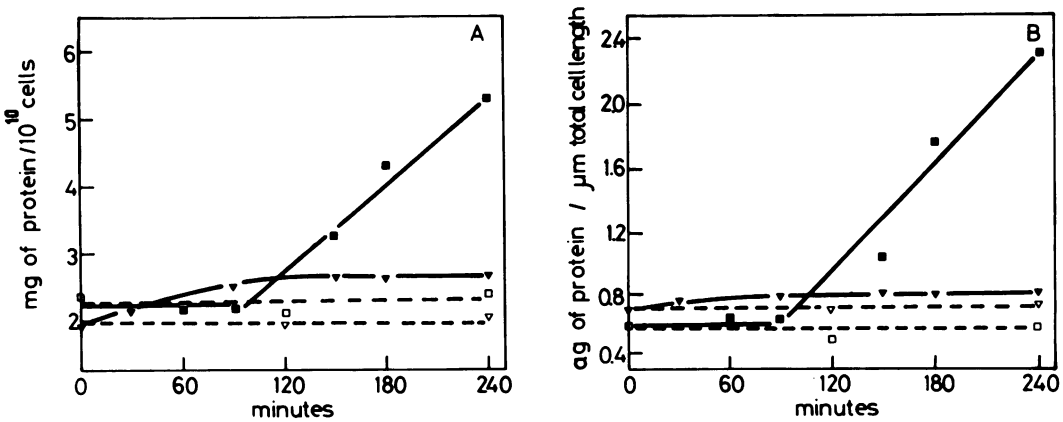

FIG. 5. *Kinetics of protein content increases. Cultures of OV-2 (wild type) and OV-25 growing exponentially at 30°C in nutrient broth plus thymine were split in two; one of each strain was left at 30°C and the other was shifted to 42°C at zero time. Samples were withdrawn at the times indicated, and the amount of protein was measured as described in the text. Protein per cell (A) and protein per total cell length (B) are shown. Triangles are OV-2 data; squares are OV-25 data. Open symbols and broken lines are cultures at 30°C; filled symbols and solid lines are cultures at 42°C.*

which the amount of protein per unit of total cell length is represented. In this case total cell length was calculated from the mean cell length of the whole population, including the filaments.

Changes in cell density in the absence of the *wee* gene product. As OV-25 cells had, at the restrictive temperature, a higher content of protein per unit of length, they should either become denser or increase their diameter to compensate for their short length. Both measurements of "Coulter volume" (Fig. 4a) and microscopic observations seemed to disfavor the last possibility. To find whether cell density changed at the restrictive temperature, we collected a sample of an OV-25 population that had been shifted from 30°C to 42°C. The sample was collected 180 min after the time of shift and centrifuged to equilibrium in a colloidal silica self-forming gradient as described in Materials and Methods. Control populations (OV-25 growing exponentially at 30°C; OV-2 growing exponentially at 30°C and 180 min after a shift to 42°C) were run under the same conditions. The results are shown in Fig. 6.

As the increase in the amount of protein per unit of length after the wild type was shifted from 30°C (τ = 45 min) to 42°C (τ = 25 min) was found to be 1.10-fold (Fig. 5b), and as the expected change in cell volume for such a shift should be 1.25-fold (assuming a behavior of radius change with growth rate similar to that described for strain B/r A; 16), it is not surprising that wild-type cells had a lower density after a shift to 42°C than at 30°C.

Cell density in OV-25 cells changed with temperature in quite a different manner. At 30°C most of the population banded at densities within the ranges found for the wild type at 30°C. At 30°C the small percentage of filaments was uniformly distributed in all the fractions. After the cells had spent 180 min at 42°C, they banded at regions of higher density, well over the values found for the wild type. The filaments remained in this sample at densities similar to that of the wild type at 42°C. When samples of OV-25 that had remained at 42°C for 240 min were run under the same conditions, most of the cells sedimented to the bottom of the gradient (results not shown).

Viability of strain OV-25 at the restrictive temperature. In contrast to the mutants altered in PBP 1Bs, in which cell elongation stops (19), OV-25 cells elongated and divided at the restrictive temperature, as we have shown above (Fig. 1). We also found that OV-25 cells did not plate successfully at the restrictive temperature. We next investigated whether OV-25 cells are viable after a shift to 42°C. Suitably

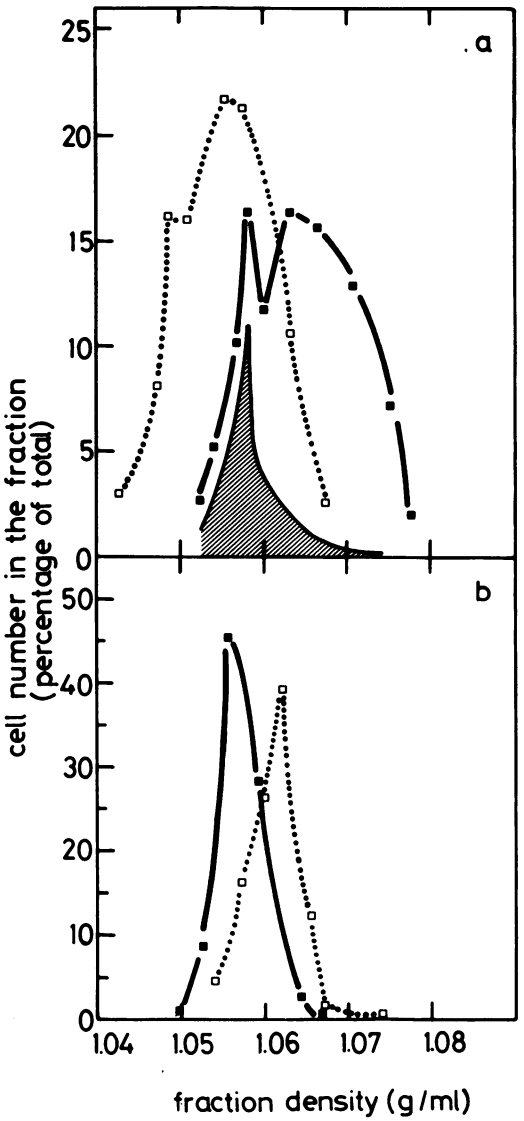

FIG. 6. *Cell density in the absence and presence of the wee gene product. Cultures of OV-25 and OV-2 growing exponentially in nutrient broth plus thymine at 30°C were split in two, one half was left at 30°C, and the other half was shifted to 42°C at zero time. After 180 min, the cells (10^9 cells in total for each subculture) were collected by centrifugation, resuspended in 0.5 ml of 10 mM phosphate buffer pH 7.6; mixed with 22 ml of 16% Ludox HS40, centrifuged to equilibrium, and fractionated as described in the text. Cell number and fraction density were measured as described in the text. (a) OV-25; (b) OV-2. Open symbols and dotted lines are results at 30°C; filled symbols and solid lines are results at 42°C. The hatched area represents the position after centrifugation of the population of filaments found in OV-25 at 42°C.*

diluted samples from a culture shifted to 42°C were plated and grown overnight at 30°C. The results in Fig. 7 indicate that, after a time in which viability at 42°C was maintained at levels similar to those found at 30°C, it dropped sharply at the same time that mean cell length decreased and protein content per total cell length increased (compare with Fig. 2 and Fig. 5).

Genetic location of the *wee* gene. Interrupted matings done on OV-25.1, a *nal* derivative of OV-25, located *wee* between the points of transfer of KL25 and KL209, that is, between min 82 and min 90 on the standard *E. coli* map (1). P1 cotransduction frequencies, calculated from experiments in which strain M6K *bgl* was used as donor and OV-25 was used as recipient, gave between 70 and 20% cotransduction with *ilv* and from 8 to 6% cotransduction with *bgl*. It was concluded, therefore, that a plausible orientation is *bgl, ilv, wee*. In all experiments stable temperature-resistant cotransductants were always selected, but temperature-sensitive *ilv*$^+$ cotransductants were found to be unstable, becoming temperature resistant after they were restreaked twice. Another case of abnormal cotransduction results in a nearby region (min 82.7) has been described by Foulds and Chai (7).

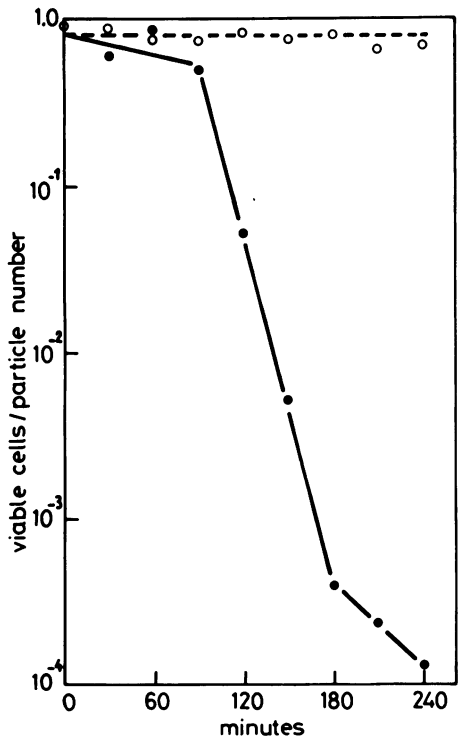

FIG. 7. *Cell viability in OV-25. A culture of OV-25 growing exponentially at 30°C in nutrient broth plus thymine was split in two at time zero. Half was left at 30°C, and the other half was shifted immediately to 42°C. Samples were withdrawn at the times indicated, particles were counted in a Coulter Counter, and suitable dilutions were plated on nutrient agar plates that were incubated overnight at 30°C. The values were corrected for the efficiency of plating found for the wild-type cells under the same conditions. Open symbols and broken line are results at 30°C; filled symbols and solid line are results at 42°C.*

DISCUSSION

Elongation in *E. coli* seems to be a complex process that proceeds at a rate proportional to the growth rate of the population, doubling the rate at a certain length (4, 6). It has been postulated as well that elongation forms a separate cell cycle pathway, being independent of DNA replication (6, 22). In spite of this, mean cell length remains almost constant at a given growth rate (6). Similarly, cell lengths at birth are strongly grouped around a central value (4). We have to conclude, then, that, at least theoretically, there are regulatory signals from the separate growth pathways that keep a rather precise control over cell parameters at division. One signal of this kind has been described for DNA replication as a protein that links replication to cell division (5, 9, 20).

Strain OV-25 under the restrictive conditions showed a normal growth pattern (Fig. 1) except for total cell length and mean cell length (Fig. 1 and 2). Mean cell length diminished after the cells were placed at 42°C. A gradual decrease in mean cell length rather than a sudden change was observed. This could be explained in either of two ways. Either the *wee* gene product disappears only by dilution, as would be expected from the amber nature of the mutation, or otherwise its absence affects not the elongation process itself but some controlling mechanism. It is very unlikely that the OV-25 phenotype at 42°C was caused just by mere dilution of the *wee* gene product, as the kinetics of total cell length increase should then change from exponential to linear upon a shift to the restrictive temperature. Moreover, if such a cause were true, an acceleration in the rate of elongation like that found after the temperature shift-up in Fig. 1 would be hard to understand.

The increase in total cell length, together with the absence of lysis at the restrictive temperature and a different genetic location, distinguishes strain OV-25 from the mutant described as affecting PBP 1Bs (19).

We would therefore explain the phenotype of OV-25 at 42°C as a defect in the control of cell elongation. The behavior of the OV-25 cells at

30°C also differed from that of the wild type; at this temperature the cells were longer than wild-type cells under the same growth conditions, although mean cell length was sensitive to changes in the growth rate of the culture (Fig. 3), as happens in the wild type. The *wee* gene product synthesized in the presence of an amber mutation suppressed by *supF*-A81(Ts) will surely be a mixture of two molecular species, as the temperature-sensitive suppressor will insert either tyrosine or glutamine in the nonsense codon (2). In this case either one or both of the *wee* molecular species will differ from the wild-type *wee* gene product. This could, to some extent, be the cause of the phenotype of OV-25 under permissive conditions; if this explanation were true, it follows that the control of cell length would also be modified at 30°C. In any case, mean cell length at 30°C does not extend beyond the values found as maximal for *E. coli* B/r A (6).

Mean cell length at 42°C approached values (2 μm), at all growth rates, that would be expected for a culture of the wild type "growing" with infinity generation time (6). This means that between birth and division the cells increase a constant amount of length (in the case of OV-25, approximately 1.71 μm) independently of their growth rate. On the other hand, although mean cell length becomes at 42°C independent of growth rate (see Fig. 3), the length doubling rate and therefore the rate of elongation are still dependent on it. The length doubling rate was calculated from the mean cell lengths and particle doubling rates plotted in Fig. 3 as follows.

Assuming that under steady-state conditions the length doubling rate and the particle doubling rate have the same value in a wild-type population, we can rewrite equation 1 as

$$\bar{L} = 2 + 2r/3 \quad (2)$$

where $r = 60/T$ is the length doubling rate, T being the time taken to double the total length of the population.

To simplify the calculation, we considered the rate of elongation, $g(x)$, to be constant through a cell cycle and to be described by

$$g(x) = RL_B/60 \quad (3)$$

where x is the length of a cell and $R = 60/\tau$, τ being the particle doubling time (generation time); this equation means that during one generation time the length at birth, L_B, is doubled. Then, as

$$L_B = \bar{L} \ln 2 \quad (4)$$

(reference 6), $g(x)$ can be calculated from the particle doubling rate, R, as

$$g(x) = \bar{L}R \ln 2/60 \quad (5)$$

and from equation 2, assuming that in steady-state populations R is equal to r,

$$g(x) = (2r + 2r^2/3) \ln 2/60 \quad (6)$$

Now we can calculate r from equations 5 and 6, as both equations for $g(x)$ should be equal in steady-state populations. The desired value for the length doubling rate, r, will then be given by the positive solutions to

$$2r^2 + 6r - 3\bar{L}R = 0 \quad (7)$$

If equation 7 is solved for those values of $\bar{L}$ and R found for OV-25 (that is, those values given in Fig. 3), a dependency of length doubling rate on particle doubling rate is found at both 30 and 42°C (see Fig. 8). It can be observed that at the restrictive temperature the length doubling rate in OV-25 is near a lower limit defined by length doubling rates at which just one unit of cell length (as defined by the length at birth of a cell

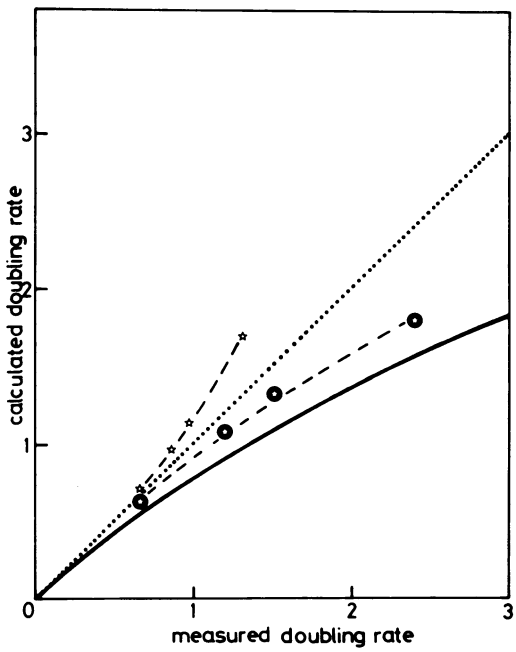

FIG. 8. *Dependency of the length doubling rate (calculated as described in the text) on the particle doubling rate (measured) in OV-25. The data shown in Fig. 3 (mean cell length, $\bar{L}$; and particle doubling rate, R) have been substituted in equation 7 (see text) to calculate the length doubling rate at 30°C (☆) and 42°C (●). The dotted line is the result expected for a wild-type strain. The solid line is the result of the calculation for a strain that adds one unit of cell length (the length at birth of a cell with infinity generation time) per cell cycle.*

with infinity generation time; reference 6) is synthesized per cell cycle.

The properties of strain OV-25 seemed, then, to derive either from a defective control of elongation or alternatively from a lack of coordination between elongation and division (i.e., cells would divide once their chromosomes are replicated and other requirements are fulfilled but before attaining the division length characteristic of their growth rate). We cannot at this time distinguish between these two possibilities. To test the predictions of both of them, we would need a temperature-sensitive *wee* allele in which inactivation of the gene product was immediate at 42°C, so that the elongation rate of synchronized populations during a single division cycle could be measured. (This strain is not yet available.) The second alternative leads to the prediction that the *D* period could possibly be shortened at 42°C in OV-25. We have been unable to test it because estimates of *D* in the parental strain OV-2, done by measuring residual division after addition of chloramphenicol or withdrawal of the required amino acids, gave values of *D* at 42°C shorter than 5 min, rendering comparison with OV-25 extremely speculative.

We found that, after a time at the restrictive temperature, protein per unit of length and density increased in OV-25 cells over the values found in the wild type. These facts enable us to formulate the cell cycle of OV-25 in the absence of the *wee* gene product at fast growing rates in the following terms: synthesis of DNA and protein and cell division proceed at a rate that is normal relative to the wild type; elongation fails to keep up its pace with those other processes, and cell diameter does not increase to values able to compensate for the decreased cell length; the cell mass is then packed in a volume smaller than that required for the rate of mass growth; and, possibly as a consequence of their increase in density over the values normally found in wild-type cells, the mutated cells lose viability and fail to plate at the restrictive temperature.

It is hoped that the identification of the *wee* gene product will help in the understanding of cell elongation; the fact that OV-25 harbors an amber allele of the gene makes such study feasible so that a more detailed description of the processes of cell elongation and their control, as well as their correlation with cell division, will be possible.

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