Division Behavior and Shape Changes in Isogenic ftsZ, ftsQ, ftsA, pnpB, and ftsE Cell Division Mutants of Escherichia coli during Temperature Shift Experiments

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Received 26 October 1987/Accepted 25 December 1987

Isogenic ftsZ, ftsQ, ftsA, pnpB, and ftsE cell division mutants of Escherichia coli were compared with their parent strain in temperature shift experiments. To improve detection of phenotypic differences in division behavior and cell shape, the strains were grown in glucose-minimal medium with a decreased osmolality (about 100 mosM). Already at the permissive temperature, all mutants, particularly the pnpB and ftsQ mutants, showed an increased average cell length and cell mass. The pnpB and ftsQ mutants also exhibited a prolonged duration of the constriction period. All strains, except ftsZ, continued to initiate new constriction sites at 42°C, suggesting the involvement of FtsZ in an early step of the constriction process. The new constrictions were blunt in ftsQ and more pronounced in ftsA and pnpB filaments, which also had elongated median constrictions. Whereas the latter strains showed a slow recovery of cell division after a shift back to the permissive temperature, ftsZ and ftsQ filaments recovered quickly. Recovery of filaments occurred in all strains by the separation of newborn cells with an average length of two times L0, the length of newborn cells at the permissive temperature. The increased size of the newborn cells could indicate that the cell division machinery recovers too slowly to create normal-sized cells. Our results indicate a phenotypic resemblance between ftsA and pnpB mutants and suggest that the cell division gene products function in the order FtsZ-FtsQ-FtsA, PBP3. The ftsE mutant continued to constrict and divide at 42°C, forming short filaments, which recovered quickly after a shift back to the permissive temperature. After prolonged growth at 42°C, chains of cells, which eventually swelled up, were formed. Although the ftsE mutant produced filaments in broth medium at the restrictive temperature, it cannot be considered a cell division mutant under the presently applied conditions.

Escherichia coli is one of the very few organisms in which the genetics of the regulation of cell division can be studied: a number of specific conditional mutants that are affected in cell division have been described (for a review, see reference 11), and an enzymatic activity of one of the gene products has been demonstrated (18). Cell division in E. coli occurs by a seemingly simple ingrowth of the envelope layers without the help of additional structures like external wall bands (15) or internal skeletal compounds (30). In spite of this simplicity, it has appeared difficult to determine the specific role of the various cell division genes. First, the composition of the envelope layers at the division site has not yet been shown to differ chemically from the rest of the envelope. As a result, we have to describe the process in morphological terms and distinguish different stages like initiation of cell wall ingrowth, formation of polar caps, and cell separation. The second reason is that inhibition of cell division can be the result of even slight perturbations of DNA synthesis (the SOS response), protein synthesis (heat shock response), secretion, and fatty acid degradation. Some of the conditional mutants which show cell division inhibition at the restrictive temperature illustrate this: tsl and tif mutants are involved in the SOS response (14, 25), the fam mutant is involved in the heat shock response (8, 39), and the secA mutant is involved in protein secretion (28). In addition, faaR mutants which have constitutive expression of enzymes involved in the beta-oxidation of fatty acids produce filaments at high growth rates (40). Screening for the so-called Fts phenotype, i.e., multinucleated filamentation, thus easily leads to the isolation of pseudo-division mutants, which indirectly affect cell division, in contrast to real cell division mutants, which have a mutation in a structural or regulatory gene involved directly in polar cap synthesis. Fortunately, the screening for real cell division mutants is sometimes facilitated by growth-rate-dependent filamentation of pseudo-division mutants. A recent example is the ftsB mutant, which has been shown to be involved in the synthesis of DNA precursors (21, 33).

The main cell division genes studied are clustered in the 2-min region of the E. coli chromosome (22), except for the ftsE gene, which has been mapped at 76 min (29). The function of the ftsQ, ftsA, and ftsE gene products is unknown, whereas the ftsZ gene product interacts with the SOS response-associated cell division inhibitor SfiA (19), which is induced after DNA damage (17). The pnpB gene encodes the peptidoglycan-synthesizing penicillin-binding protein 3 (PBP3) (32). All the above genes encode proteins that are required to initiate, form, and complete constrictions and have been proposed to act in a morphogenetic pathway (2, 11). However, the phenotypes of all of the cell division mutants thought to be specific (ftsZ, ftsQ, ftsA, pnpB, ftsE) are remarkably uniform: filaments with or without partial constrictions. Previously, no mutants had been detected that show, for instance, a prolonged constriction period, an aberrant precision of the location of the division site, or a different shape of the polar caps at the nonpermissive condition.

In this work, we have measured the effect of the above mutations on the division behavior and shape of cells with the same genetic background and under standardized growth conditions.

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conditions: we used minimal medium with a decreased osmolality, which allowed a relatively slow growth rate, to detect even small phenotypic differences that might help to arrange the mutants in a morphogenetic pathway of cell division.

**MATERIALS AND METHODS**

**Bacterial strains.** All strains used in this study are listed in Table 1.

**Media and growth conditions.** Broth containing 1% tryptone (Difco Laboratories), 0.5% yeast extract (Difco), and 0.5% NaCl was used as rich medium (TY). TY agar consisted of TY broth supplemented with 1.5% agar (Difco). Minimal medium contained 1.58 g of K$_2$HPO$_4$ · 3H$_2$O, 0.74 g of KH$_2$PO$_4$, 1.05 g of (NH$_4$)$_2$SO$_4$, 0.10 g of MgSO$_4$ · 7H$_2$O, 0.3 mg of FeSO$_4$ · 7H$_2$O, 7.1 mg of Ca(NO$_3$)$_2$ · 4H$_2$O, 1 mg of thiamine, 5 g of glucose, and 100 mg of lysine per liter. In this medium the usual concentration of phosphate salts was halved to reach an osmolality of 100 mosM; the osmolality of the usual minimal medium is 200 to 300 mosM (46). Constancy of average cell mass of strains cultured in this medium was taken as indication that steady-state growth could be achieved (44). If necessary, tetracycline (12.5 μg/ml) and kanamycin (50 μg/ml) were added. For temperature shift experiments, cultures growing in the steady state at 28°C in a water bath shaker were diluted fourfold in prewarmed medium at 42°C. After one or two mass doublings at 42°C, the culture was shifted back to 28°C by diluting twice in prewarmed medium. Absorbance was measured with a 300 T-1 spectrophotometer (Gilford Instrument Laboratories Inc.). Cell numbers were determined by using a Coulter Counter with a 30-μm-diameter orifice.

**Genetic techniques.** P1 vir-mediated transduction was carried out as described by Miller (23). Strains were tested for temperature sensitivity on TY plates without NaCl at 42°C and on TY plates with 0.5% NaCl at 30°C. Temperature-sensitive transductants were compared with the original mutants and could be complemented by the same plasmids.

**Electron microscopy.** Samples were fixed with 0.1% OsO$_4$. One-fifth volume of 5 × concentrated TY broth was added to the fixed cells to promote spreading of the cells during agar filtration (45). Cell sizes were measured from electron micrographs as described previously (38).

**RESULTS**

**Differences between isogenic cell division mutants at the permissive temperature.** We constructed a set of isogenic cell division mutants and determined several physiological and morphological parameters in temperature shift experiments (Fig. 1). Because it is important for such comparative studies to grow all mutants under steady-state conditions, we only used minimal medium cultures, in which steady state growth is achieved more easily than in rich medium (unpublished observations). We also used minimal medium because it is appropriate for radioactive dianimopinic acid incorporation experiments (42), which cannot be performed in broth or media containing Casamino Acids. In addition, we lowered the salt concentration of the medium to prevent salt suppression of the cell division mutations (29).

At the permissive temperature, all mutants except the fitsA6 mutant appeared to be larger in mass and length than the parent strain (Table 2). This applied especially to the fitsQ and pbP mutants, which were up to 35% longer than the parent strain. As a result, all mutants except fitsA6 showed a more elongated cell shape (L/2R in Table 2; Fig. 2). No significant change in the precision of cell division as defined by the coefficient of variation of the K(L) distribution (37) was found (Table 2).

The percentage of constricting cells was higher in all mutants except fitsZ and fitsA6. As a result, the constriction

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**TABLE 1. E. coli K-12 strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC500</td>
<td>F&lt;sup&gt;+&lt;/sup&gt; araD139 Δ(argF-lac)U169 rpsL150 ffbB5301 ptsF25 deoC1 rbsR relA1 lysA1</td>
<td>33</td>
</tr>
<tr>
<td>LMC502</td>
<td>LMC500 leu::Tn5</td>
<td>P1(6976) × LMC500</td>
</tr>
<tr>
<td>LMC509</td>
<td>LMC502 leu&lt;sup&gt;+&lt;/sup&gt; fitsZ84(Ts)</td>
<td>P1(JFL100) × LMC502</td>
</tr>
<tr>
<td>LMC510</td>
<td>LMC502 leu&lt;sup&gt;+&lt;/sup&gt; pbpB2158(Ts)</td>
<td>P1(LMC360) × LMC502</td>
</tr>
<tr>
<td>LMC511</td>
<td>LMC502 leu&lt;sup&gt;+&lt;/sup&gt; fitsA10(Ts)</td>
<td>P1(LMC361) × LMC502</td>
</tr>
<tr>
<td>LMC512</td>
<td>LMC502 leu&lt;sup&gt;+&lt;/sup&gt; fitsA1882(Ts)</td>
<td>P1(LMC359) × LMC502</td>
</tr>
<tr>
<td>LMC515</td>
<td>LMC505 fitsE1181(Ts) zyg-1::Tn10</td>
<td>P1(LA5697) × LMC500</td>
</tr>
<tr>
<td>LMC589</td>
<td>LMC505 fitsA6(Ts) zab::Tn10</td>
<td>J. R. Walker (1)</td>
</tr>
<tr>
<td>AX621</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsA1882(Ts) thr-1 leuB6 Δ(gpt-proA)62 hisG4 thi-1 argE3 lacY1 galK2 xyl-5 mtl-1 ara-14 tsx-33 rfbD1 rpsL31 supE44 λ&lt;sup&gt;−&lt;/sup&gt; rac mgl-507 kdgK51</td>
<td>J. R. Walker (1)</td>
</tr>
<tr>
<td>AX655</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; pbpB2158(Ts) thr-1 leuB6 Δ(gpt-proA)62 hisG4 thi-1 argE3 lacY1 galK2 xyl-5 mtl-1 ara-14 tsx-33 rfbD1 rpsL31 supE44 λ&lt;sup&gt;−&lt;/sup&gt; rac mgl-507 kdgK51</td>
<td>J. R. Walker (1)</td>
</tr>
<tr>
<td>TFK10</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsA10(Ts) thr leu thyF codA thyC lacY fhuA supE</td>
<td>J. R. Walker (1)</td>
</tr>
<tr>
<td>TOE-1</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsQ1(Ts) thyA leu proA his thi argE lacY galK xyl mtl ara tsx rpsL supE</td>
<td>J. R. Walker (1)</td>
</tr>
<tr>
<td>MFT1181</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsE1181(Ts) thyA1 thr-1 leuB6 thi-1 argH1 gpI trp-l lacY1 gal-6 metL-2 xyl-7 malA1 (K&lt;sup&gt;−&lt;/sup&gt;) ara-13 rpsL2 fhuA2 λ&lt;sup&gt;−&lt;/sup&gt; supE44 fic mel</td>
<td>J. R. Walker (29)</td>
</tr>
<tr>
<td>LA5697</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsA6(Ts) araD139 Δ(arg-lac)U169 rpsL150 ffbB5301 ptsF25 deoC1 rbsR relA1 zab::Tn10</td>
<td>W. Boos (5)</td>
</tr>
<tr>
<td>JGC109</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; strB1 zyg-1::Tn10 argG6 metB his-1 leu-6 thyA3 metL-2 xyl-7 malA1 gal-6 lacY1 rpsL104 fhuA2 tax λ&lt;sup&gt;−&lt;/sup&gt; n&lt;sup&gt;−&lt;/sup&gt; supE44</td>
<td>J. R. Walker (33)</td>
</tr>
<tr>
<td>6976</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; pro thi leu::Tn5</td>
<td>J. R. Walker (22)</td>
</tr>
<tr>
<td>JFL100</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; fitsZ84(Ts) thy thyA leu proA his thi argE lacZ125(Am) galU142(Am) tyr(Ts)</td>
<td>J. R. Walker (23)</td>
</tr>
<tr>
<td>LMC359</td>
<td>AX621 leu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>P1(LMC360) × AX621</td>
</tr>
<tr>
<td>LMC360</td>
<td>AX655 leu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>P1(LMC361) × AX655</td>
</tr>
<tr>
<td>LMC361</td>
<td>TKF10 leu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>P1(LMC362) × TKF10</td>
</tr>
<tr>
<td>LMC380</td>
<td>MFT1181 zyg-1::Tn10</td>
<td>P1(JGC109) × MFT1181</td>
</tr>
<tr>
<td>LMC385</td>
<td>TOE-1 leu&lt;sup&gt;+&lt;/sup&gt;</td>
<td>P1(LMC300) × TOE-1</td>
</tr>
</tbody>
</table>
FIG. 1. Comparison of the effect of a temperature shift on cell division in isogenic strains: parent strain LMC500 (A), pbpB mutant LMC510 (B), ftsZ mutant LMC509 (C), ftsE mutant LMC515 (D), ftsQ mutant LMC531 (E), and ftsA1882 mutant LMC512 (F) grown in minimal medium. At time zero, cultures growing at the steady state were shifted from 28 to 42°C. After one mass doubling time at 42°C, the cultures were shifted back to 28°C. Absorbance (○) and cell number (△) were determined at intervals. Average cell mass (△) is expressed in arbitrary units calculated by dividing the absorbance by the cell number. Arrows indicate times at which samples for electron microscopy were taken.
or T period increased from 31 min in the parent strain to about 45 min in the fisQ and phpB mutants (Table 3). Mutant cells were longer and still showed round polar caps, except in the phpB mutant, which had more pointed polar caps (Fig. 2B). Thus, the cell division mutations were already partly manifested at the permissive temperature by a change in cell shape and by a lengthening of the constriction period.

**Effect of the temperature shift on the parent strain.** The temperature shift from 28 to 42°C caused a transient increase in average cell mass in the parent strain (Fig. 1A), which has been observed previously (10, 39). Within two mass doubling times at 42°C, the parent regained the same average cell mass as at 28°C during steady-state growth (data not shown). It seems unlikely that this is caused by the induction and expression of the heat shock genes as suggested by Tsuchido et al. (39), because htpR mutants, in which the expression of the heat shock genes is prevented at the restrictive temperature, still form filaments (8, 39; unpublished results). This excludes the possibility of a cell division inhibitor regulated by htpR. Alternatively, transient filamentation at high temperature could result from a slower increase in the rate of DNA synthesis relative to mass synthesis. Such a difference in adaptation would cause a temporary increase in average cell mass. The absence of the heat shock response in htpR mutants might abolish the reversibility of filamentation after a temperature shift, in a way similar to the way a lon mutation prevents recovery of filamentation after UV irradiation (24).

**Effect of the temperature shift on the mutants.** When shifted to 42°C, all strains showed an increase in the rate of mass synthesis (Fig. 1). The average cell mass of the mutants increased as they started to produce filaments (Fig. 3). However, the fitsE mutant eventually formed small chains of swollen cells (Fig. 3D).

Without exception, the mutants showed a considerable amount of residual division during 20 to 40 min after the shift to the restrictive temperature, whereafter the cell number remained constant. Of the constricting cells at 28°C, 16 to 76% were able to finish cell division at the restrictive temperature (Table 3). The fraction of constricting cells at 28°C that finished cell division at 42°C could indicate the step in the constriction process that was inactivated at 42°C. In two independent experiments, the phpB mutant did show the smallest amount of residual division, suggesting that PBP3 is involved in a later stage of the division process. Residual division in the fitsZ mutant was on an average higher than that of the others (Table 3), suggesting a role for fitsZ in an early stage of constriction.

Constrictions which were not terminated at 42°C persisted in most strains (Table 3). The shape of persisting constrictions in fitsZ and fisQ mutants (Fig. 3C and E) did not seem to change, although occasionally an extended constriction was observed in fisQ filaments. In the phpB mutant, 76% of

<table>
<thead>
<tr>
<th>Strain</th>
<th>Doubling time (min)</th>
<th>Avg cell mass (avg Aavg cell)</th>
<th>Avg cell lengthb (μm)</th>
<th>Length of newborn cellc (μm)</th>
<th>Cell shapea (L/D)</th>
<th>CVKLD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC500 (parent)</td>
<td>84-88</td>
<td>2.0-2.1</td>
<td>1.9-2.2</td>
<td>1.4</td>
<td>2.3</td>
<td>4.5</td>
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<tr>
<td>fitsZ4</td>
<td>88-75</td>
<td>2.6-2.2</td>
<td>2.3-2.2</td>
<td>1.6</td>
<td>2.8</td>
<td>5.6</td>
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<tr>
<td>fitsQ</td>
<td>86-82</td>
<td>2.9-3.0</td>
<td>2.7-2.9</td>
<td>1.9</td>
<td>3.6</td>
<td>4.7</td>
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<tr>
<td>fitsA1882</td>
<td>80-83</td>
<td>2.4-2.2</td>
<td>2.3-2.2</td>
<td>1.6</td>
<td>2.8</td>
<td>5.5</td>
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<tr>
<td>fitsA10</td>
<td>85</td>
<td>2.2</td>
<td>2.1</td>
<td>1.4</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>fitsA6</td>
<td>75</td>
<td>2.0</td>
<td>1.9</td>
<td>1.4</td>
<td>2.2</td>
<td>ND</td>
</tr>
<tr>
<td>phpB2158</td>
<td>88-74</td>
<td>3.3-3.2</td>
<td>2.9-3.1</td>
<td>2.0</td>
<td>4.2</td>
<td>5.6</td>
</tr>
<tr>
<td>fitsE1181</td>
<td>80-76</td>
<td>2.4-2.2</td>
<td>2.4-2.1</td>
<td>1.6</td>
<td>3.0</td>
<td>4.8</td>
</tr>
</tbody>
</table>

a Results are from cells grown in glucose-minimal medium (100 mosM) at 28°C in two independent experiments. Cell parameters are based on measurements of over 500 cells per strain.

b The variation coefficient of the average cell length varied from 22 to 40.

c The average length of newborn cells was calculated with the formula $L_0 = T/2\ln 2$ (44).

L is the average cell length; $2R$ is average cell diameter.

* Variation coefficient of the distribution of the ratio of the length of prospective daughter cell to the length of the dividing cell (37). ND, Not determined.
the original constrictions could not be terminated, and they
were extended to blunt constrictions (necks) (Fig. 3B).
These extended constrictions, which resemble those in cells
treated with cephalothin (6) or furazlocillin (27) and those in
a sefA mutant (26), were also observed in all three ftsA
mutants (Fig. 3F). Newly initiated constrictions at 42°C
accounted for 24 to 56% of the constrictions present at the
restrictive temperature in all mutants except the ftsZ mutant
(Table 3). Constrictions initiated by the mutants at 42°C were
blunt compared with those of the parent strain (Fig. 3A) or
with those initiated at 28°C (Fig. 2). These constrictions
were blunt, probably because progress of the constriction
in the mutants was slowed down at 42°C, while elongation of
the cells continued.

Effect of a shift back to the permissive temperature. After
one mass doubling time at 42°C, the cultures were shifted
back to 28°C. All strains showed a temporary arrest of mass
synthesis (Fig. 1). Thereafter, the growth rate increased to
the normal steady-state rate at 28°C. Except for the duration
of the recovery of the filaments, which was relatively long
for the ppxB and ftsA mutants (Fig. 1B and F), no difference
in the mode of recovery was found. Like the ppxB mutant,
the other mutants formed constrictions at a distance between
one and two times Lo (the average length of the newborn
cell) from the cell poles (Fig. 4). Because incubation for one
mass doubling time at 42°C resulted in filaments that were
not long enough to distinguish between cells born during
recovery at 28°C and short, nonconstricting cells already
present at 42°C (e.g., formed by residual division), we also
studied division recovery after an incubation for two mass
doubling times at 42°C. The average length of the newborn
ppxB cells themselves was not Lo, as it would be during
steady-state growth at the permissive temperature, but
rather two times Lo (Fig. 5) (47). This placement of the
constriction site at a length larger than Lo and division of
newborn cells at a length of two times Lo was also observed in
ftsZ and ftsA mutants and confirmed former results of
others (1, 36). A possible explanation for the formation of
enlarged daughter cells could be that the constriction ma-
achinery recovers too slowly to separate newborn cells with
an average length of Lo. Alternatively, the recovering cells
could prefer to divide between nucleoids which have been
segregated for a longer time. The presence of still replicating
or segregating chromosomes could inhibit cell division at Lo.

The recovery of cell division in the ppxB mutant was
sometimes accompanied by the formation of aberrant con-
strictions near the cell poles, whereby minicell-like cells
were formed (Fig. 3, insert). In the ftsA1882 mutant, similar
cells were observed with lower frequency. The aberrant
constrictions might indicate that the cells had difficulty with
the recovery of division at the blunt constrictions, which
would explain the relatively long recovery times in the
ppxB and ftsA mutants (Fig. 1B and F).

DISCUSSION

Application of steady-state growth conditions in minimal
medium enabled us to detect subtle differences between the
various isogenic cell division mutants, both at the permissive
temperature and after temperature shifts. If the different
gene products would function sequentially in a so-called
morphogenetic pathway, our observations argue for the
following sequence: FtsZ-FtsQ-FtsA, PBP3. In this se-
cquence, FtsE is not considered because the mutant did not
produce filaments in minimal medium at 42°C. Instead, ftsE
formed small chains of somewhat swollen cells (Fig. 3D).
Although the use of minimal medium with a higher osmo-
ality (300 mosM) suppressed swelling of the ftsE cells, it
did not restore filamentation as found during growth in rich
medium at 42°C (data not shown). We consider this filamenta-
tion to be an effect of the higher growth rate and are not
convinced, therefore, that the ftsE gene is directly involved
in the constriction process.

In principle, cell division mutants can be divided in the
same classes as used for DNA synthesis mutants: quick-
stop, slow-stop, and reduced-rate mutants (31). Whereas the
classification of dna(Ts) mutants depends on how the shift to
the restrictive temperature affects the incorporation of ra-
dioactive thymine, the progress of constriction formation is
the selected parameter for classification of cell division
mutants. Quick-stop division mutants can be thought of as
carrying a mutation in a gene involved in the progress of the
constriction and display an immediate inhibition of cell
division as the protein denatures on a shift to the restrictive
temperature. Slow-stop mutants carry a mutation in a gene
involved in the initiation of the constriction. Although initi-
ation no longer occurs, the existing enzyme complexes
forming the constriction terminate the process and cause

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**TABLE 3. Division behavior of temperature-sensitive cell division mutants and their parent strain after a temperature shift from 28 to 42°C**

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of constricting cells (A)</th>
<th>Constriction period (min) (T)</th>
<th>Total division % of:</th>
<th>% of cells showing a median constriction</th>
<th>% of newly initiated constrictions (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC500 (parent)</td>
<td>29–29</td>
<td>31–32</td>
<td>65–53</td>
<td>31–34</td>
<td>0–3</td>
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<tr>
<td>ftsQ1</td>
<td>45–47</td>
<td>46–46</td>
<td>21–11</td>
<td>47–23</td>
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<td>ftsA10</td>
<td>32</td>
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<td>8</td>
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<td>70</td>
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<tr>
<td>ppxB2158</td>
<td>42–43</td>
<td>45–38</td>
<td>10–7</td>
<td>24–16</td>
<td>41–40</td>
</tr>
</tbody>
</table>

* Results are from two independent experiments. The following formulas were used (letters refer to column headings): $T = \ln(1 + A) / \ln2 \times T_D$ (44); $B = [(cell number_{28} /cell number_{42}) - 1] \times 100%; C = B/A \times 100%; D = [(A - B)/(100 + B)] \times 100%; F = E - D.

$D$ represents the fraction of cells that had a constriction before the shift and did not finish division at 42°C, i.e., that contained persisting constrictions. The denominator $(100 + B)$ is used to correct for the increase of the population at 42°C.
residual division. Reduced-rate mutants are assumed to have a partially incapacitated cell division gene product, so that division continues at a slower rate.

At the permissive temperature, all cell division mutants studied showed the reduced-rate phenotype: the cell shape was changed, and the constriction process was prolonged. Especially, *ftsQ* and *pbpB* mutants initiated constrictions much earlier than all others. The constriction machinery was probably less active and operating at a reduced rate, possibly due to a partial inactivation of the gene product already at the permissive temperature. However, at the restrictive temperature, all mutants in this study showed residual division, as expected for the slow-stop phenotype. Although the amount of residual division is expected to be allele dependent, the amount of residual division in the *ftsZ* and *ftsE* mutants was relatively high and that in the *pbpB* and *ftsA10* mutants was low in comparison with the *ftsQ* and the other *ftsA* mutants (Table 3). If the amount of residual division would be related to the step of the constriction process that was inhibited, then it is tempting to suggest that FtsZ is involved in the constriction process before FtsQ and FtsA and that these are followed by PBP3. No consistent relationship was observed between the T period and the amount of residual division (Table 3).

In previous studies, cell division mutants have been arranged in a morphogenetic pathway by their ability to initiate

FIG. 3. Electron micrographs of isogenic cell division mutants and their parent grown two mass doubling times in minimal medium at 42°C: parent strain LMC500 (A), *pbpB* mutant LMC510 (B), *ftsZ* mutant LMC509 (C), *ftsE* mutant LMC515 (D), *ftsQ* mutant LMC531 (E), and *ftsA1882* mutant LMC512 (F). The insert in B shows the aberrant constrictions of *pbpB* mutant LMC510 formed during recovery at 28°C after incubation for two mass doublings at 42°C. Bar, 1 μm.
structured double mutants, which had a rodA(Ts) or pbpA(Ts) mutation in addition to a fisA(Ts), fisQ(Ts), or pbpB(Ts) mutation and which were able to form constrictions at 42°C. Based on the latter result, we think it somewhat premature to assign to FtsA a function after PBP3 and still assume that they are acting in a similar step of the cell division process. An indication that FtsA could interact with PBP3 is provided by Tormo et al. (34), who showed that the PBP3 in a fisA mutant was unable to bind radioactive ampicillin at 42°C. Begg et al. (4) found evidence for another interaction of PBP3 with RodA, which could be only the beginning of the elucidation of a complex constriction machinery.

Although the influence of the growth rate on the phenotype of the presumed cell division mutants has been illustrated already by the fisB (33) and fisE (this work) mutants, we have found another example. The presence of the presumed division mutation fisM (12) in LMC500 grown in minimal medium did not result in filamentation under restrictive circumstances (unpublished results). The function of fisM in Weigle reactivation and its regulation of lexA (12) suggest a role in SOS-associated repair. The map position of the fisM gene near leu (12) does not exclude the possibility that fisM is an allele of polB (7, 16) and dinA (20).

Characterization of more cell division mutants under the standard conditions defined in this paper is necessary if we wish to distinguish genes specifically involved in the cell division process from the pseudo-division genes functioning in processes which on perturbation affect cell division.

ACKNOWLEDGMENTS

This work was supported by the Foundation for Fundamental Biological Research, which is subsidized by the Netherlands Organization for the Advancement of Pure Research.

We thank J. R. Walker for advice and stimulating discussions during the course of this work; J. R. Walker, W. Boos, and G. R. Drapeau for providing bacterial strains; J. Leutscher for drawings on January 20, 2018 by guest http://jb.asm.org/ Downloaded from