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Inhibition of Cell Division Initiation by an Imbalance in the Ratio of FtsA to FtsZ

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Elevated levels of FtsA protein block cell division at a very early stage, similar to that caused by inhibition of the action of FtsZ. In contrast, overexpression of FtsA and FtsZ together does not block division. A specific ratio of FtsA to FtsZ protein, therefore, is required for cell division.

The first sign of division in cells of *Escherichia coli* is a circumferential constriction, associated with the formation of an internal ring of FtsZ protein (2). Further constriction, and the formation of a peptidoglycan cross wall, requires the action of other proteins, including FtsA. At restrictive temperatures, mutants which either cannot make FtsA protein or make a thermolabile form grow into long filaments with regularly spaced constrictions (1, 5). These constrictions are particularly easy to see in *ftsA*(Ts) *rodA*(Ts) or *ftsA*(Ts) *pbpA*(Ts) double mutants, which form swollen cells with deep constrictions at 42°C (1). In contrast, *ftsZ*(Ts) mutants at 42°C (like cells in which the action of FtsZ has been inhibited by the action of the SOS-induced SulA inhibitor) grow as unconstricted filaments, and *ftsZ*(Ts) *rodA*(Ts) or *ftsZ*(Ts) *pbpA*(Ts) double mutants grow into unconstricted swollen cells at this temperature. This has been interpreted to mean that cells lacking active FtsZ protein are blocked at the earliest known stage in cell division (1).

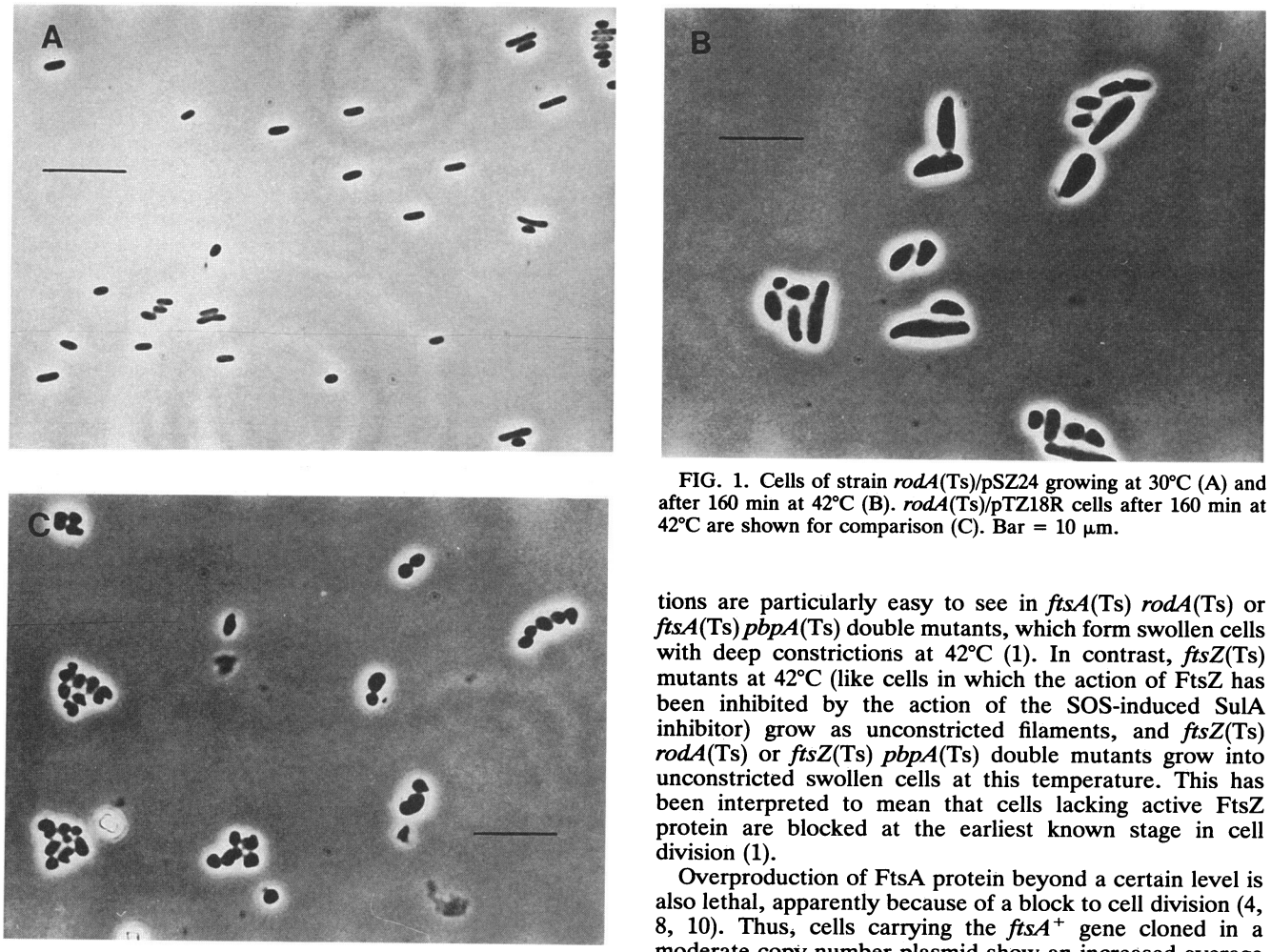

FIG. 1. Cells of strain *rodA*(Ts)/pSZ24 growing at 30°C (A) and after 160 min at 42°C (B). *rodA*(Ts)/pTZ18R cells after 160 min at 42°C are shown for comparison (C). Bar = 10 μm.

Overproduction of FtsA protein beyond a certain level is also lethal, apparently because of a block to cell division (4, 8, 10). Thus, cells carrying the *ftsA*$^+$ gene cloned in a moderate-copy-number plasmid show an increased average cell length at 37°C but a much greater inhibition of cell division at 42°C. The plasmid pSZ24 carries a 2.4-kb *Eco*RI chromosomal fragment comprising the *ftsQ* and *ftsA* genes cloned in pTZ18R (4). Complementation tests with *ftsQ*(Ts)

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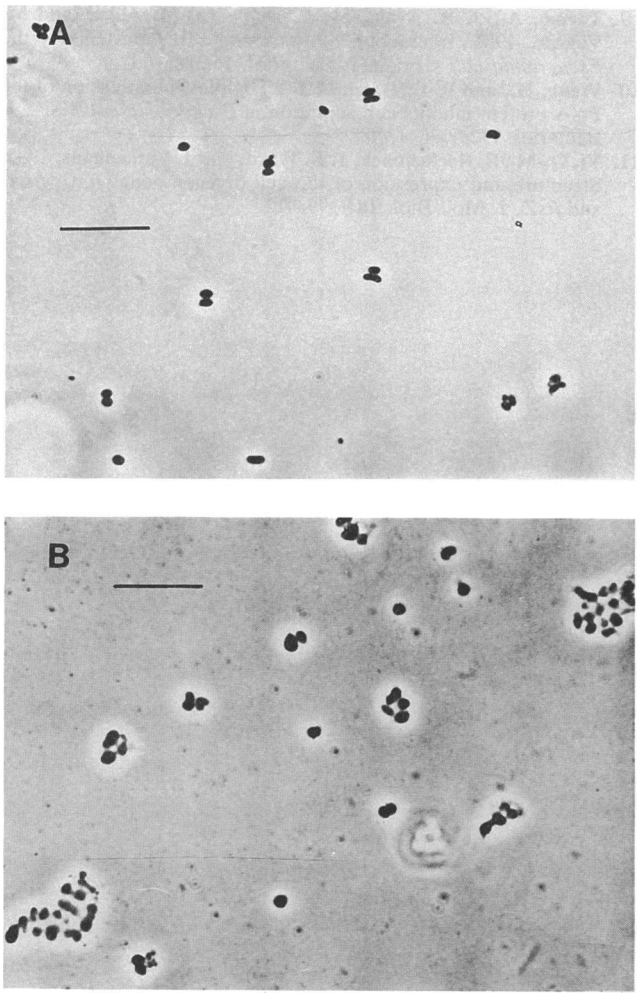

FIG. 2. Cells of strain *rodA*(Ts)/pZAQ growing at 30°C (A) and 42°C (B). Bar = 10 μm.

and *ftsA*(Ts) mutants show that the plasmid expresses *ftsA* but not *ftsQ* (because the promoters upstream of the *ftsQ* reading frame are not included in this fragment) (7, 11), and so the partial block to cell division must result from expression of the cloned *ftsA* gene (in agreement with earlier findings). We noticed that the filamentous cells produced in this way did not appear to have the regularly spaced constrictions characteristic of cells blocked in the FtsA-dependent step in cell division. To demonstrate this clearly, we introduced pSZ24 into *rodA*(Ts) and *pbpA*(Ts) mutants. As shown in Fig. 1, the *rodA*(Ts)/pSZ24 cells grew as normal rods at 30°C but as unconstricted swollen cells at 42°C; the *pbpA*(Ts)/pSZ24 cells showed a similar phenotype (data not shown). We conclude that overproduction of FtsA blocks cell division at a very early stage.

Because a deficiency in FtsZ protein blocks division at a similar early stage, we wondered whether a coordinate increase in the levels of both FtsA and FtsZ proteins would prevent this block in cell division. In accord with this prediction, we found that the plasmid pZAQ, which carries the complete *ftsQ ftsA ftsZ* sequence, does not cause filamentation at 42°C and that *rodA*(Ts) and *pbpA*(Ts) mutants carrying this plasmid are able to divide normally as spheres at 42°C (Fig. 2). To ensure that pZAQ overproduced FtsA protein at a level comparable with that of pSZ24, we deleted the *ftsZ* gene from it to create a new plasmid. This construct, which complemented *ftsA* and *ftsQ* mutants but no longer rescued *ftsZ84*, produced effects identical to those of pSZ24 in wild-type cells and in a *rodA* mutant. It seems likely, therefore, that overproduction of the FtsA protein interferes with the action of FtsZ in the initiation of cell division.

The recent report by Bi and Lutkenhaus (2) shows that FtsZ protein, which is present at up to 20,000 molecules per cell, is generally distributed throughout the cytoplasm in nondividing cells but that it condenses into a circumferential ring at the cell membrane at the site and time at which cell division begins. This internal protein ring is presumably the cause of the regularly spaced constrictions seen in cells which are blocked in subsequent stages of septum formation (e.g., *ftsA*, *ftsQ*, or *ftsI* mutants). The absence of constrictions in cells which are overproducing FtsA protein therefore suggests that the formation of the FtsZ ring is prevented.

A possible explanation of our observation is that FtsA protein normally interacts directly with FtsZ protein but that, when FtsA is present in excess, this prevents the normal assembly of the Z ring. In this respect, it is interesting that FtsA protein is normally produced at only a few hundred molecules per cell, so that the normal ratio of FtsZ to FtsA is of the order of 100:1. *ftsA* and *ftsZ* are adjacent genes with no transcription terminators between them; however, their strongly differential levels of expression appear to be assured by a combination of transcriptional and translational controls. Thus, *ftsZ* is transcribed from a number of promoters, some of which are upstream of *ftsA* but others of which are within the *ftsA* coding frame (7, 8, 11) so that *ftsZ* is expected to be transcribed more frequently than *ftsA*. In addition, translation of *ftsA* mRNA is very inefficient relative to *ftsZ* mRNA (5a, 6). Because plasmids which are simultaneously overexpressing both FtsA and FtsZ do not block cell division, it now seems clear that it is the differential level of expression of FtsA and FtsZ proteins which is essential for normal cell division.

Finally, FtsA is a predominantly cytoplasmic protein (like FtsZ) but it has been reported that an intermediate-density membrane fraction, supposed to correspond to the inner-outer membrane fusion zone at the septum, is enriched in FtsA protein (3). FtsA and FtsZ proteins may therefore be in the same septal structure in dividing cells. A septal localization for FtsA has also been inferred from morphological studies on *ftsA* mutants (9).

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