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The Balance between Different Peptidoglycan Precursors Determines Whether *Escherichia coli* Cells Will Elongate or Divide

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The *rodA*(Sui) mutation allows cell division to take place at 42°C in *ftsI23* mutant cells, which produce a thermolabile penicillin-binding protein 3 (PBP3, the septation-specific peptidoglycan transpeptidase). We show here that the mutation in *rodA* is a single-base change from a glutamine to a chain termination (amber) codon, and that an amber suppressor (*supE*) present in the strain restores the ability to produce a reduced level of normal RodA protein. The reduced level of RodA is accompanied by an increase in the levels of two other proteins (PBP2 and PBP5) encoded by genes in the *rodA* operon. We show that an increased level of PBP5 is by itself sufficient to restore cell division to *ftsI23* cells at 42°C. Two other treatments were found to restore division capacity to the mutant: an increase in PBP6 (which is a D-alanine carboxypeptidase like PBP5) or suitable concentrations of D-cycloserine. All of the above treatments have the effect of reducing the number of pentapeptide side chains in peptidoglycan and increasing the number of tripeptides. We conclude that the effect of the *rodA*(Sui) mutation is to indirectly increase the availability of tripeptide side chains, which are used preferentially by PBP3 as acceptors in transpeptidation. A change in the proportions of different kinds of peptide side chain in the peptidoglycan can therefore determine whether cells will divide.

The shape of *Escherichia coli* cells is determined by the shape of the peptidoglycan layer or sacculus, which consists of a hollow cylinder with hemispherical poles. During the cell cycle, two morphogenetic processes alternate: cell elongation, by increase in the length of the cylindrical portion of the sacculus, and septation, with the formation of two new hemispherical poles in the old cell center. The morphogenetic proteins responsible for the maintenance of the cylindrical shape during the elongation phase include RodA and penicillin-binding protein 2 (PBP2), whereas a number of proteins, including PBP3, carry out septation (7, 8, 15). The membrane proteins PBP2 and RodA together carry out peptidoglycan transpeptidation and transglycosylation, as also does PBP3 (perhaps in combination with the recently described FtsW membrane protein [10, 15]). The PBP2-RodA and PBP3-FtsW pairs of membrane proteins therefore carry out alternating morphogenetic modifications of the growing sacculus and are responsible for the multiplication of *E. coli* cells as rods with hemispherical poles.

In a previous paper (3) we showed that the temperature-dependent block to division in cells with a mutation (*ftsI23*) in the gene coding for PBP3 is reversed by a second mutation [*rodA*(Sui)] in the gene coding for the RodA protein. In that paper we speculated that the PBP3 and RodA proteins might interact directly in the cell membrane, such that a mutational change in one could be compensated for by a change in the other. However, we have now sequenced the mutated *rodA* gene and found that there is a single-base change that converts the CAG codon of Gln-111 to a TAG amber stop codon. Since the strain used also carries an amber suppressor, *supE* (which recognizes UAG as a Gln codon), the only effect of the *rodA*(Sui) mutation is to produce a reduced level of wild-type RodA protein. Therefore no conformational change in RodA (compensating for the change in PBP3) has in fact taken place.

In the present paper we show how the reduced level of RodA protein can reverse the division block in mutants that produce a temperature-sensitive PBP3. We found that the *rodA*(Sui) mutation had no effect on the thermostability of the mutant PBP3 protein but that it was accompanied by an increase in the levels of both PBP2 and PBP5, which are also coded for by genes in the *rodA* operon (16, 32). We show that an increase in PBP5 is by itself sufficient to reverse the cell division block in *ftsI23* cells. Since PBP5 is a D-alanine carboxypeptidase I, which catalyzes the removal of the terminal D-alanine from pentapeptide side chains in the peptidoglycan, an increase in this enzyme will increase the proportion of tetrapeptide to pentapeptide side chains in the peptidoglycan. In support of this interpretation, we find that increased levels of PBP6, another D-alanine carboxypeptidase I, also reverses the division block in *ftsI23* cells. Evidence has been presented by others (2, 4, 22) that tripeptide side chains, in which both terminal D-alanine residues have been removed, are the favored acceptor for transpeptidation catalyzed by PBP3. An increase in the amount of tetrapeptide could lead to an increase in tripeptide through the action of carboxypeptidase II (2). No gene for carboxypeptidase II has yet been identified, but an increase in the proportion of tripeptide side chains can also be achieved by partial inhibition of the D-alanine-adding reactions with the antibiotic D-cycloserine (22). In agreement with our hypothesis, we found that appropriate levels of D-cycloserine could also reverse the division block in *ftsI23* cells. Finally, we show that division in suitably suppressed *ftsI23* mutant cells at 42°C is, in fact, still dependent on the residual activity of PBP3, because division is still sensitive to specific inhibitors of PBP3.

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TABLE 1. *E. coli* K-12 strains, bacteriophages, and plasmids

Strain, phage, or plasmid	Genotype or characteristics	Source or reference
E. coli		
AB2497	*thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE37 thyA12 thyR14*	Laboratory collection
TOE23	Like AB2497, but *ftsI23*(Ts)	3
I4	Like TOE23, but *rodA*(Sui)	3
KJB1	Like TOE23, but *rodA*(Sui), obtained by P1 transduction via I4	This study
SJC21	*rodA51*(Ts) *recA56 srlC300*::Tn*10 thi-1 his-4 purB15 proA2 mtl-1 xyl-5 galK2 lacY rpsL35*	1
AT1325lip9	*thi-1 his-4 purB15 proA2 mtl-1 xyl-5 galK2 lacY1 lip-9 rpsL35 supE44*	Laboratory collection
KEN222	Like AT1325lip9, but *rodA*(Sui) *lip*$^+$, obtained by P1 transduction via I4	3
MV1184	*ara* Δ(*lac-pro*) *rpsL thi* φ80*lacIZ*M15 Δ(*srl-recA*)*306*::Tn*10*(F′ *proAB lacI*^{q} *Z*M15 *traD36*)	34
Bacteriophages		
P1		Laboratory collection
M13K07	Helper phage for the production of phage particles from pUC118 and pUC119	34
λ *c*Its857		11
λ *c*Its857 *E*am1009	Like λ *c*Its857, but amber mutation 1009 in gene *E*	11
λ *c*Its857 *E*am1010	Like λ *c*Its857, but amber mutation 1010 in gene *E*	11
λ *c*Its857 *E*am1012	Like λ *c*Its857, but amber mutation 1012 in gene *E*	11
Plasmids		
pUC13	Ap^r	17
pHSG398	Cm^r	33
pUC118/119	Ap^r	34
pMA102	Ap^r, pBR322 carrying the *pbpA* and *rodA* genes	2
pST1	Ap^r, pUC13 carrying the *pbpA* and *rodA*(Sui) genes	This study
pSA100	Cm^r, pHSG398 carrying the *rodA*(Sui) gene	This study
pBS47	Km^r, carrying the *pbpA* gene: derivative of pSC105	31
pBS59	Km^r, carrying the *dacA* gene: derivative of pSC105	31
pBS110	Ap^r, carrying the *dacC* gene: derivative of pSC105	6
pSU55	Ap^r, the 1.6-kb *Bam*HI-*Eco*RI fragment carrying *dacA* from pBS59, under P_{tac} control in pJF118HE	This study
pBK18-1	Ap^r, 1.8-kb *Sma*I-*Eco*RI fragment carrying *dacB* in pUC18	W. Keck

In summary, our hypothesis is that the amber mutation in *rodA* (in the presence of *supE*) results in a lower level of RodA protein, which (directly or indirectly) leads to derepression of the *rodA* operon and increased amounts of PBP5. This increases the availability of tetrapeptide substrates for carboxypeptidase II, which produces the tripeptide acceptor chains used by PBP3. This increase in substrate concentration compensates for the reduced PBP3 activity at 42°C in *ftsI23* cells and allows septation to take place.

Our findings therefore support the hypothesis (4) that the switch from cell elongation to cell divisions may be brought about, in part, by a change in the relative availabilities of the different peptide side chains favored by two competing morphogenetic systems (PBP2-RodA and PBP3-FtsW). This model for division control has been supported by the earlier findings that the activity of carboxypeptidase II fluctuates during the cell cycle and reaches its maximum at the time of septation (4, 18, 19); and that increase in carboxypeptidase Is (PBP5 and PBP6) causes cells to lose their rod shape and become spherical (14). D-Cycloserine also causes cells to become spherical (22). It has been proposed that the spherical shape results from overactivity of the PBP3-dependent septation system at the expense of the PBP2-RodA-dependent system, which maintains the cylindrical shape of cells during the elongation phase. Our results are fully in accord with these interpretations.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. The strains of *E. coli* K-12, bacteriophage, and plasmids used in this work are listed in Table 1.

Media and growth conditions. Cells were grown with vigorous shaking in Difco L broth (LB) or Oxoid nutrient broth no. 2 (NB); 1.5% agar was added for solid media. Ampicillin (100 μg/ml), chloramphenicol (10 μg/ml), and kanamycin (20 μg/ml) were added to media as required.

Molecular cloning and DNA hybridization. Cloning and hybridization methods were based on those of Maniatis et al. (13). Chromosomal DNA was isolated by the procedure of Rodriguez and Tait (26). Plasmid DNA was prepared by a modified alkaline extraction method (13). Transformation of *E. coli* cells was carried out by the method of Kushner (12).

Cloning the *rodA*(Sui) gene. Chromosomal DNA of strain KJB1 was digested with *Sma*I and *Bam*HI and then separated by electrophoresis on 0.8% agarose. The *pbpA* and *rodA* genes are found in a single 4.3-kb *Sma*I-*Bam*HI fragment (1), and therefore fragments of about this size were extracted from the gel and ligated into pUC13 (17) that had been digested with *Sma*I and *Bam*HI. MV1184 (34) cells were transformed with the plasmids. Transformants that grew at 37°C on a nitrocellulose membrane on LB agar containing ampicillin were replicated onto another nitrocellulose membrane, which was then submitted to colony hybridization (13). The 1.1-kb *Mlu*I-*Bam*HI fragment con-

taining the larger part of the *rodA* gene was obtained from plasmid pMA102 (1) and used as a hybridization probe. A DNA CHEMIPROBE kit (Takara Co., Kyoto, Japan) was used for modification and detection of the probe (23). In this way we obtained a plasmid, pST1, that contained the *pbpA* and *rodA*(Sui) genes. The 1.6-kb *Kpn*I-*Bam*HI fragment containing *rodA*(Sui) was subcloned from pST1 into the *Kpn*I-*Bam*HI site of pHSG398 (33) to produce pSA100.

DNA sequencing. The restriction fragments derived from the 1.6-kb *Kpn*I-*Bam*HI fragment were cloned into the multiple cloning sites of pUC118 (34) and pUC119 (34). Single-stranded DNA was prepared after infection with helper phage M13K07 (34) and sequenced by the dideoxy-chain termination method (27).

Southern hybridization. Chromosomal DNA from strains KJB1 and TOE23 were digested with *Kpn*I and *Bam*HI, separated by electrophoresis on 0.8% agarose, and stained with ethidium bromide. DNA fragments were blotted from the agarose gel onto a nylon membrane (Hybond-M; Amersham) (24).

DNA hybridization was carried out with a ^{32}P-labeled oligodeoxynucleotide (13) in the presence of a competitor oligodeoxyribonucleotide (20). The oligodeoxyribonucleotides were synthesized with an Applied Biosystems model 380B DNA synthesizer.

Detection of PBPs. Cells of strains AB2497 (*ftsI*$^+$), TOE23 (*ftsI23*), and KJB1 [*ftsI23 rodA*(Sui)] were grown to the late-exponential phase in LB medium at 30°C, and crude cell envelopes were prepared as described previously (28). The cell envelopes were incubated at 30°C for 10 min with [^{3}H]penicillin (final concentration, 20 μg/ml, [27 Ci/mmol]) after preincubation at 42°C for 0, 1, 2.5, or 5 min. The samples were fractionated on a 12% sodium dodecyl sulfate–polyacrylamide gel, and the PBPs were detected by fluorography (28).

RESULTS

Sequence of *rodA*(Sui). The *rodA*(Sui) gene was cloned into plasmid vectors from chromosomal DNA of strain KJB1, and its sequence was compared with that of the wild-type *rodA*$^+$ allele. Only one nucleotide difference was found: the C at nucleotide 331 of *rodA*$^+$ (16) was replaced by T in *rodA*(Sui) (Fig. 1). The Sui mutation was therefore shown to be a change from CAG (the codon for Gln-111 of the RodA protein) to TAG (the amber termination codon).

A ^{32}P-labeled oligonucleotide containing the mutated base (5′-TGTTCGTTTTTAGCCGTC-3′) was hybridized to chromosomal DNA in the presence of an unlabeled wild-type sequence oligonucleotide competitor. This showed that the mutation was present in an appropriately sized *Kpn*I-*Bam*HI restriction fragment of chromosomal DNA from KJB1 but not in that from the parent strain TOE23 (data not shown).

Confirmation that strain KJB1 contains the amber suppressor *supE*. A number of amber mutations in gene *E* of bacteriophage lambda are known to be suppressed by a suppressor mutant of glutamine-tRNA, *supE*, but not by *supD* (serine-tRNA) or *supF* (tyrosine-tRNA) (11). Strain AB2497, from which TOE23 and KJB1 were derived, carries the amber suppressor allele *supE37*. To confirm that KJB1 still carried this amber suppressor, three mutant phages carrying different *supE*-suppressible mutations in the *E* gene (*E*am1009, *E*am1010, *E*am1012) were used to test for the presence of *supE* in KJB1. Strain KJB1 was sensitive to all three mutant phages, as well as to the *E*$^+$ parent phage. This confirms that this strain carries *supE37*. The presence of the mutant glutamine-tRNA will therefore replace glutamine at the mutated UAG codon in *rodA*(Sui) mRNA with low efficiency, resulting in the production by KJB1 of a reduced level of normal RodA protein.

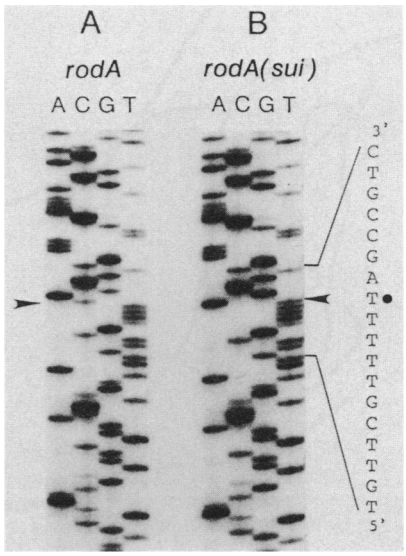

FIG. 1. Nucleotide sequence of the *rodA*(Sui) allele, in comparison with that of the wild-type *rodA*$^+$ gene, in the region of the mutation site. The C at nucleotide 331 in the *rodA*$^+$ allele (13) has been changed to T in the *rodA*(Sui) allele, as indicated by arrowheads.

Effect of *rodA*(Sui) on PBPs. As previously reported (3), KJB1 cells [*ftsI23 rodA*(Sui) *supE*] are able to divide at 42°C, unlike the parent TOE23 cells (*ftsI23 supE*). Figure 2 shows the pattern of PBPs in the cell membranes of AB2497 (*ftsI*$^+$ *supE*; the parent of TOE23), TOE23, and KJB1. The cells were grown at 30°C; the membranes were prepared and preincubated at 42°C for 0, 1, 2.5, or 5 min before labeling with [^{3}H]penicillin for 10 min at 30°C (see Materials and Methods). AB2497 and TOE23 cells showed a similar pattern of PBPs, except that PBP3 in TOE23 was reduced in amount even at 30°C and disappeared rapidly on incubation at 42°C. This confirmed that the *ftsI23* mutation altered the thermostability of PBP3, something that had not previously been

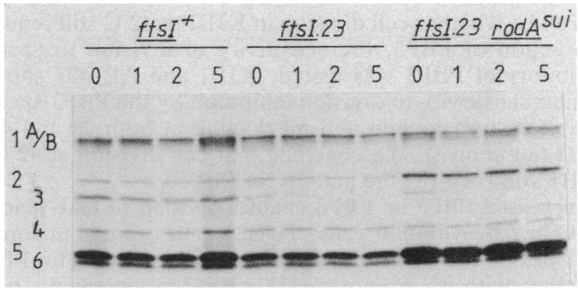

FIG. 2. PBPs in strains AB2497 (*ftsI*$^+$), TOE23 (*ftsI23*), and KJB1 [*ftsI23 rodA*(Sui)]. The cells were grown to the late-exponential phase in LB at 30°C, and crude cell envelopes were prepared as described previously (24). The cell envelopes were incubated at 30°C for 10 min with [^{3}H]benzylpenicillin (final concentration, 20 μg/ml [27 Ci/mmol]) after preincubation at 42°C for 0, 1, 2.5, or 5 min. The samples were fractionated on a 12% sodium dodecyl sulfate–polyacrylamide gel, and the PBPs were detected by fluorography (24).

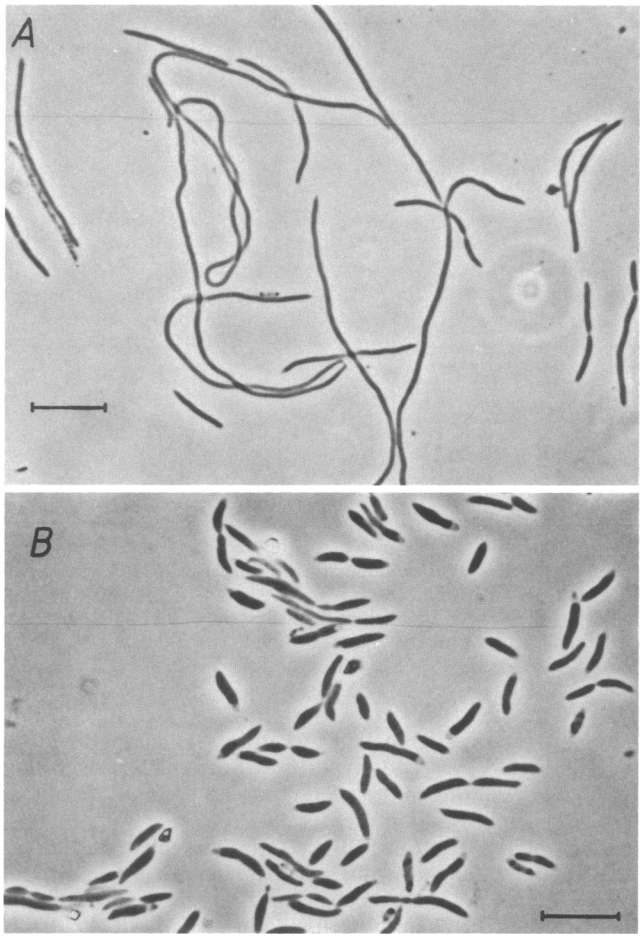

FIG. 3. (A) Cells of strain TOE23 (*ftsI23 rodA*$^+$ *supE*) after growth at 42°C. (B) Cells of strain TOE23 carrying the plasmid pBS59 (*dacA*$^+$) after growth at 42°C. The cells were grown on NB agar plates overnight at 42°C. Only cells carrying the plasmid were able to form colonies. Bar, 10 μm.

demonstrated directly. The presence of the *rodA*(Sui) mutation had no detectable effect on the level or stability of PBP3; however, this mutation was associated with a marked increase in the levels of two other PBPs, PBP2 and PBP5 (Fig. 2).

To test whether cell division in KJB1 at 42°C still required the action of PBP3, the sensitivity of division to specific inhibitors of PBP3 was tested. KJB1 and AB2497 showed similar sensitivity to division inhibition by the PBP3-specific inhibitors furazlocillin and mezlocillin at both 30 and 42°C (data not shown). We conclude that cell division at 42°C in KJB1 still requires the activity of PBP3.

Increased PBP5 or PBP6 enables division to take place at 42°C in *ftsI23* mutant cells. TOE23 cells were transformed with pBS47, which carries the *pbpA*$^+$ gene (coding for PBP2) together with its promoter (31). pBS47 complements *pbpA* chromosomal mutations but had no effect on the temperature sensitivity of division in TOE23. In contrast, pBS59, carrying the 1.6-kb *Bam*HI-*Eco*RI restriction fragment with the *dacA*$^+$ (PBP5) gene and its promoter, permitted TOE23 cells to form colonies on plates at 42°C. Figure 3A and B show TOE23 cells and TOE23(pBS59) cells, respectively, growing on agar at 42°C. The presence of the plasmid has restored the ability to divide to the PBP3 mutant cells. [The *ftsI23*(pBS59) cells, although dividing, are also of increased diameter, but *ftsI*$^+$(pBS59) cells are almost spherical (18).] Another plasmid (pSU55) was constructed in which the 1.6-kb *dacA* fragment was placed under the control of a Ptac promoter in a high-copy-number ColE1-derived plasmid. This plasmid allowed even better division of TOE23 cells at 42°C, but the addition of increasing concentrations of an inducer (isopropyl-β-D-thiogalactopyranoside) did not further improve division, although it did cause the cells to become increasingly swollen. High levels of isopropyl-β-D-thiogalactopyranoside were lethal. Increased levels of PBP5 are therefore sufficient to restore cell division to TOE23 mutant cells at 42°C.

The *dacA* and *pbpA* genes are tightly linked to *rodA*, and all three genes may be part of a single transcriptional unit (although internal promoters are also present) (16, 30). The increased levels of their products in *rodA*(Sui) cells may therefore result from derepression of the whole operon in response to reduced levels of RodA protein. There are other genes in this operon (32), but they are not PBPs and were not visible in the penicillin-labeled gels used here. The restriction fragments cloned in the pBS47 and pBS59 plasmids should not have expressed any of these other membrane proteins; but, to demonstrate that it is increased carboxypeptidase I activity which restores the ability to divide to TOE23 cells, we transformed the mutant with pBS110. This plasmid expresses the *dacC* gene coding for PBP6 (5, 6), which is also a carboxypeptidase I. Cells carrying pBS110 produce about five times the normal level of PBP6 and become swollen to some extent. In accord with our hypothesis, we found that TOE23(pBS110) cells were also able to divide at 42°C (data not shown).

PBP4 also has D-alanine carboxypeptidase activity in vitro, although its in vivo role is thought to be different from that of PBP5 and PBP6. Its function may be as an endopeptidase (21). We found that plasmid pBK18-1, which expresses the *dacB* gene coding for PBP4, did not restore the ability to divide at 42°C in KJB1 cells. This supports the idea that the role of PBP4 in vivo is different from that of PBP5 and PBP6.

We conclude that KJB1 [*ftsI23 rodA*(Sui) *supE*] cells are able to divide at 42°C because of an increased level of the carboxypeptidase I enzyme PBP5.

Restoration of cell division in TOE23 cells by D-cycloserine. The D-alanine carboxypeptidase enzymes PBP5 and PBP6 cleave the terminal D-alanine from un-cross-linked pentapeptide side chains in the peptidoglycan and therefore increase the number of tetrapeptide side chains (14). These in turn will be cleaved by D-alanine carboxypeptidase II to produce tripeptide side chains (4). The net effect of increasing either PBP5 or PBP6 might therefore be to increase the availability of tripeptide side chains at the expense of pentapeptide chains. An increased level of carboxypeptidase II might therefore also be able to restore division to PBP3-deficient cells. Because the gene for carboxypeptidase II has not yet been identified, we were not able to test the effect of increasing this enzyme. However, we were able to treat the cells in such a way as to increase the amount of tripeptide side chains by another route. D-Alanine dipeptide is produced from L-alanine by a racemase and a ligase and then added to UDP-*N*-acetylmuramyl tripeptide to form the pentapeptide precursor of mature peptidoglycan (21). The activities of the racemase and ligase can be inhibited specifically by D-cycloserine, and cells treated with this drug have been shown to have peptidoglycan with an increased proportion of tripeptide side chains (22).

We therefore plated cells of AB2497 and TOE23 on plates

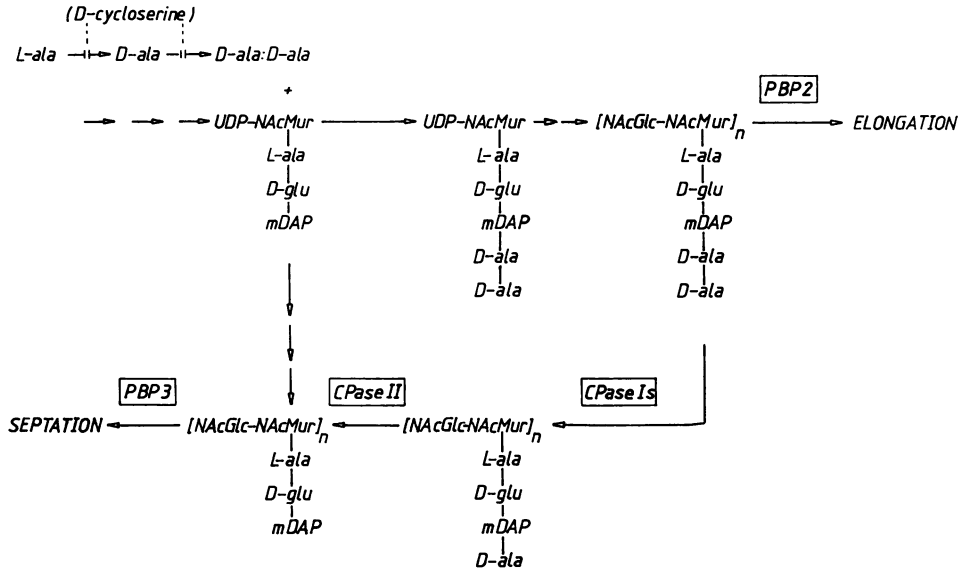

FIG. 4. Schematic outline of peptidoglycan synthesis and the proposed switch between cell elongation and cell division. UDP-*N*-acetylmuramyl tripeptide is converted to pentapeptide by the addition of D-alanine–D-alanine dipeptide. Subsequent reactions link these units together, alternating with *N*-acetylglucosamine, in long glycan chains, which are then cross-linked by transpeptidation to form the peptidoglycan sacculus. Pentapeptides (or possibly tetrapeptides) are always required as donors in transpeptidation reactions (21, 22). In this scheme, synthesis of peptidoglycan in the form of a cylindrical sacculus (causing cells to grow by elongation) requires the action of PBP2, which utilizes tetrapeptides (or possibly pentapeptides) as acceptors in transpeptidation. The formation of a septum requires transpeptidation carried out by PBP3, which utilizes tripeptides as acceptors. The availability of tripeptides depends on both (i) the production of D-alanine–D-alanine dipeptide and (ii) the activity of D-alanine carboxypeptidase I and II, which together remove the terminal D-alanine residues from pentapeptide side chains. D-Cycloserine inhibits the synthesis of D-alanine–D-alanine dipeptide and causes the production of peptidoglycan with increased numbers of tripeptide side chains (22). It is proposed that the switch from cell elongation to septation (and vice versa) depends on changes in the availability of tripeptide acceptors brought about by periodic changes in the relative activities of carboxypeptidases (and possibly of enzymes involved in the production of D-alanine–D-alanine dipeptide).

containing a range of concentrations of D-cycloserine and incubated then at 30 and 42°C. We found that certain concentrations of the drug did, indeed, allow TOE23 cells to form colonies at 42°C. The most striking result was obtained with 25 μg/ml, which inhibited colony formation by TOE23 at 30°C but permitted good colony formation at 42°C, thus reversing the normal temperature sensitivity of this strain.

DISCUSSION

We interpret our results to show that a block to cell division caused by greatly reduced levels of the septation-specific peptidoglycan transpeptidase PBP3 can be reversed by an increase in the proportion of peptidoglycan with tripeptide side chains. This is in agreement with earlier evidence that such tripeptides are the preferred acceptor residues in transpeptidation reactions carried out by PBP3 (4, 22). In cells with normal levels of PBP3, such an increase in the availability of tripeptide (brought about either by increased levels of PBP5 or PBP6 [14, 22] or by partial inhibition by D-cycloserine of the addition of D-alanine dipeptide [22]) results in an increase in the activity of PBP3-dependent peptidoglycan synthesis, at the expense of that carried out by the PBP2-RodA system, with the consequent conversion of rod-shaped cells to coccal forms. All of the treatments [*rodA*(Sui) mutation, increase in PBP5 or PBP6, treatment with D-cycloserine] that we have found to restore cell division to the PBP3-deficient mutant (TOE23) also convert cells with normal levels of PBP3 into coccal forms.

These observations suggest that the cell cycle of *E. coli*, consisting as it does of alternating phases of cell elongation and septation, depends on a regularly shifting balance between the activities of two competing morphogenetic systems. We have shown that this balance can be altered by changes in the availability of different peptide side chains. That this may also be part of the normal regulation of the growth and division cycle has been suggested by observations that the activities of carboxypeptidases change during the cell cycle (4, 18, 19). Carboxypeptidase II activity has been reported to reach a peak before cell division in normal cells and also to show a sharp increase in activity just before the resumption of division in cells of a division mutant that had been returned to 30°C after a period of growth without division at 42°C (2). Further investigation of the cycle-dependent regulation of carboxypeptidase II may therefore provide new insight into the way in which bacterial cells switch from cell elongation into division.

Figure 4 shows (schematically) the way in which the two morphogenetic systems may be connected. Glycan chains with pentapeptide side chains are synthesized in several steps from a UDP-*N*-acetylmuramyl tripeptide precursor (21). We imagine that such pentapeptide side chains are used preferentially in transpeptidation carried out by PBP2 and that this somehow results in growth by elongation of the cylindrical cell (28). D-Alanine carboxypeptidase I (PBP5 and PBP6) removes terminal D-alanine residues from pentapeptides to produce tetrapeptide side chains, which are in turn acted on by D-alanine carboxypeptidase II to produce tripeptide side chains (21). Such tripeptides are the preferred acceptors for transpeptidation carried out by PBP3, which is responsible for septum formation (4). A periodic increase in carboxypeptidase II activity (2) may perhaps be responsible for the normal switch from cell elongation to cell division in

growing cells, but in our experiments we achieve a similar effect by increasing the amount of carboxypeptidase I and thus increasing the amount of substrate (tetrapeptide side chains) for carboxypeptidase II. In wild-type cells with normal levels of PBP3, such an increase in substrate availability causes cells to become spherical, presumably because the PBP2-dependent morphogenetic system is being deprived of substrates relative to the PBP3-dependent septum-forming system (14); but in *ftsI23* mutant cells with greatly reduced amounts of PBP3 at 42°C, the increase in tripeptide relative to pentapeptide shifts the balance of morphogenetic activity from cell elongation into septation. The reduction in RodA levels in cells carrying the Sui mutation indirectly brings about this shift in substrate proportions and therefore restores the ability to divide in *ftsI23* cells at 42°C.

Spratt (28) has estimated that normal cells contain only about 50 molecules of PBP3. In Fig. 2 it is clear that the number of active PBP3 molecules in TOE23 at 42°C must be very much less than this, and yet these cells, when provided with suitable amounts of PBP3-specific substrate, are able to carry out cell division. It will be interesting to find out how so few molecules of PBP3 can be sufficient to carry out the formation of a structure as large as a bacterial septum.

Pisabarro et al. (22) have shown that D-cycloserine, which is a specific inhibitor of the L-alanine racemase and D-alanine:D-alanine ligase enzymes (Fig. 4), also causes an increase in the number of tripeptide side chains in the peptidoglycan. High levels of the drug are lethal because a minimum number of pentapeptide (or tetrapeptide) side chains is required for donors in transpeptidation, but at lower concentrations the drug causes cells with normal PBP3 levels to become rounded or spherical, just like an increase in the level of PBP5 or PBP6 (22). It is the fact that appropriate concentrations of D-cycloserine are able to restore division capacity to *ftsI23* cells at 42°C that confirms that it is an increase in tripeptide, rather than in tetrapeptide, that is required to restore the balance between cell elongation and septation in the mutant cells.

Our experiments show the probable way in which the Sui mutation in the *rodA* gene causes the restoration of cell division in PBP3-deficient cells. However, this does not by itself show the way in which the switch from cell elongation to division comes about during the normal cell cycle. The reported cycle- and division-dependent changes in carboxypeptidase II activity (2) suggest that this may be part of the mechanism of the switch, an idea which is fully supported by our observations here. However, it is also possible that a decrease in the activities of any or all of the three enzymes that bring about the addition of the D-alanine dipeptide (L-alanine racemase, D-alanine:D-alanine ligase and the dipeptide-adding enzyme) could be part of the mechanism of the switch. The cycle-dependent increase in carboxypeptidase II activity seems to depend on the prior action of the FtsA protein (the mutation causing the temperature-sensitive cell division block in the BUG6 strain used in the work described in reference 2 has now been shown to be in the *ftsA* gene [25]). Expression of PBP3 is also under negative control by the product of the *mreB* gene (35) and positive control by the *ftsH* gene (9). The evident complexities of the control system governing the switch into (and out of) cell division are now increased by our observation that the RodA protein, required for the elongation phase of the cycle, negatively regulates the production of PBP5, which is part of the septation system. All of these interactions will provide a rich field of investigation for future studies of the cell division cycle, but for the moment it seems reasonable to suppose that periodic shifts in the availability of peptidoglycan with different kinds of peptide side chains may be part of the mechanism of the cycle.

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