The analysis of cell division and cell wall synthesis genes reveals mutationally inactivated *ftsQ* and *mraY* in a protoplast-type L-form of *Escherichia coli*

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Abstract

Cell division and cell wall synthesis are tightly linked cellular processes for bacterial growth. A protoplast-type L-form *Escherichia coli*, strain LW1655F$^+$, indicated that bacteria can divide without assembling a cell wall. However, the molecular basis of its phenotype remained unknown. To establish a first phenotype–genotype correlation, we analyzed its *dcw* locus, and other genes involved in division of *E. coli*. The analysis revealed defective *ftsQ* and *mraY* genes, truncated by a nonsense and a frame-shift mutation, respectively. Missense mutations were determined in the *ftsA* and *ftsW* products yielding amino-acid replacements at conserved positions. FtsQ and MraY, obviously nonfunctional in the L-form, are essential for cell division and cell wall synthesis, respectively, in all bacteria with a peptidoglycan-based cell wall. LW1655F$^+$ is able to survive their loss-of-functions. This points to compensatory mechanisms for cell division in the absence of murein sacculus formation. Hence, this L-form represents an interesting model to investigate the plasticity of cell division in *E. coli*, and to demonstrate how concepts fundamental for bacterial life can be bypassed.

Introduction

Cell division and cell wall synthesis are highly linked processes integrated into the metabolism for bacterial growth (Nanninga, 1998). In *Escherichia coli,* cell division takes place at the midcell after the chromosome is duplicated and segregated into two daughter nucleoids. After formation of a ring-like structure (Z-ring) composed of FtsZ, other cell division-specific Fts-proteins localize at the constriction site in a striking linear hierarchy (Goehring & Beckwith, 2005). There, they co-ordinate the invagination of the cytoplasmic membrane (CM), and the inward growth of the murein (peptidoglycan) sacculus, and the outer membrane (OM) layer. Although significant progress has been made in the identification of cell division proteins and their localization to their site of action, little is known about their true functional role, and the factors which are involved to co-ordinate specifically the growth of the cell wall during the late steps of cell division (Goehring & Beckwith, 2005).

Here, we examined a mutant derived from the *E. coli* K-12 laboratory strain (Fig. 1), which had irreversibly lost the ability to form a cell wall. The mutant had been isolated by long-term cultivation of its cell wall-bearing parental strain W1655F$^+$ in the presence of penicillin and sucrose without any mutagenic treatments, until a protoplast-type L-form emerged (Schuhmann & Taubeneck, 1969). The mutant, designated LW1655F$^+$, is able to grow surrounded only by the CM. Electron microscopical studies have shown spherical cells, lacking the typical components of the Gram-negative envelope, e.g. flagellae and fimbriae, the OM, the murein layer, and the periplasmic space (Schuhmann & Taubeneck, 1969; Gumpert *et al.*, 1971). The lacking envelope components account for osmotic fragility, for resistance against cell wall-active antibiotics (e.g. ampicillin), for sensitivity towards inhibitors which cannot pass the OM, for resistance to coliphage infection, and for the inability of the L-form to mobilize genetic material via conjugation (Schuhmann & Taubeneck, 1969; Gumpert *et al.*, 1971, 2002). Growth of the L-form is confined to complex media, indicating strict dependency on rich nutrient supply. Since its isolation 35 years ago, no reversion to the cell wall-bearing phenotype has been observed. Neither penicillin as selective agent nor osmotic stabilizers are required to maintain the mutant phenotype (Gumpert *et al.*, 2002).

Considering the importance of the cell wall for essential cellular processes such as cell division, morphogenesis, or homeostasis (Nanninga, 1998), it is puzzling how *E. coli* is able to grow without assembling a proper cell wall. Despite previous reports indicating that LW1655F$^+$ may have bypassed the link of cell division and cell wall synthesis, the genetic basis of its phenotype was never investigated. We hypothesized that its stable phenotype is associated with changes in the genes responsible for cell division and for cell wall synthesis, compared with normal *E. coli*.

After confirming the identity of the L-form as an *E. coli* K-12 descendant, we performed a comparative sequence analysis of selected cell division and cell wall synthesis genes with the reference genes of *E. coli* K-12 MG1655F$^-$ (Blattner *et al.*, 1997). The results obtained provide strong evidence that mutant LW1655F$^+$ offers a chance to investigate major deviations of bacterial division in the future.

Table 1. Markers of the parent strain W1655F$^+$ determined in LW1655F$^+$

Gene	Protein variation	DNA changes in LW1655F^{+*}
16S rRNA		99.93 % identity†; A to C mismatch at pos. 80 is present in the L-form and its parent (reference is the *rrnE* of *Escherichia coli* K-12 MG1655F$^-$)
relA	Protein disruption	1337 bp insertion at position 261
spoT	84QD insertion	CAGGAT insertion at position 252
	K662N	A → C at position 1986
creC	R77P	G → C at position 230
	Frame shift	A deletion at position 1048
metB	Frame shift	GC deletion at position 141–142

*Position counted from the start triplet of the respective gene.
†1517 bp of the 16S rRNA gene of the L-form and parent W1655F$^+$ were amplified and compared with the *rrnE*.

Materials and methods

Bacterial strains and growth conditions

Escherichia coli strains LW1655F$^+$ and W1655F$^+$ were cultivated in brain heart infusion (BHI) broth (Difco). Thirty milliliters BHI broth were inoculated with 3 mL (LW1655F$^+$) and with 0.3 mL (K-12 or W1655F$^+$) of overnight cultures and shaken on a rotary shaker (200 r.p.m., 37 °C). For plating, media were solidified with 1.2% (w/v) Bacto Agar (Difco) and supplemented with 10% (v/v) horse serum.

Nucleotide sequence determination and analysis

Genomic DNA was extracted with QiaTip columns according to the lysis and purification protocols recommended by the manufacturer (Qiagen, FRG) and used as the template to amplify polymerase chain reaction (PCR) products. Oligonucleotides of 17–25 nucleotides (nt) deduced from the *E. coli* K-12 MG1655 genome (Acc. no. U00096) served as primers. Taq and PFU polymerase (PeqLab, FRG) were used according to instructions of the supplier. Long-range PCR was performed with Platinum Taq High Fidelity DNA Polymerase-Mix according to the instruction of the supplier (Invitrogen, FRG). Primers were designed within the GAP software (Staden *et al.*, 2000) and synthesized at MWG-Biotech (Ebersberg, FRG). After PCR product purification (Qiagen), sequencing was carried out with the dye terminator method on ABI 377 and ABI3700 sequencers, according to the instructions of the manufacturer (Applied Biosystems). The nucleotide sequence of both strands of the PCR products were determined in all cases. Sequence data were processed and edited with the GAP software version 4.6 (Staden *et al.*, 2000). Nucleotide sequence and primary structure analysis was performed with BLAST (Altschul

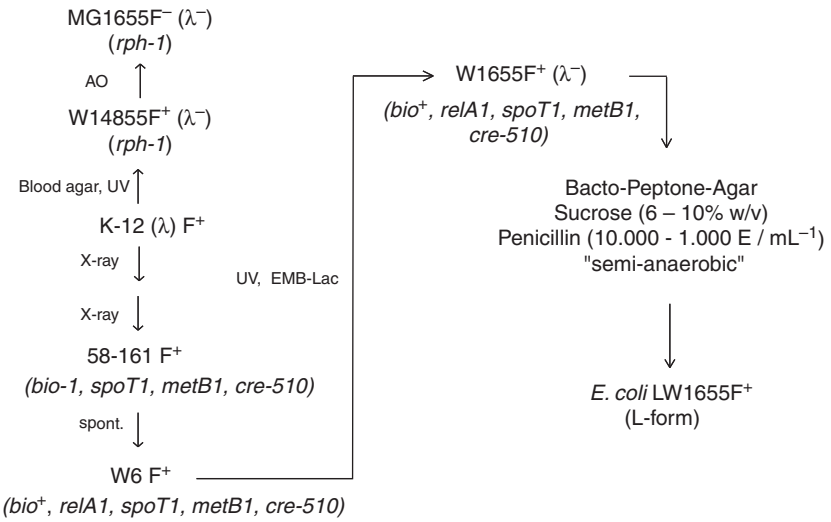

Fig. 1. Pedigree of *Escherichia coli* K12-derived strains and lineage of the protoplast-type L-form LW1655F$^+$. AO, acridine orange for curing the fertility-plasmid F; UV, ultraviolet treatment; spont., spontaneous mutation; EMB-Lac, eosin-methyleneblue lactose selection; X-ray, treatment with γ-irradiation; λ$^-$, λ, absence, and presence of phage λ, respectively. The conditions of LW1655F$^+$ isolation (Schuhmann & Taubeneck, 1969) are indicated.

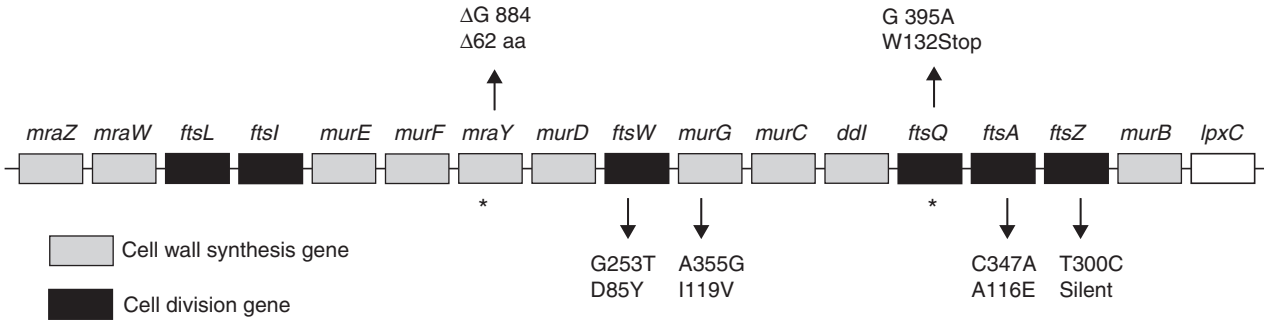

Fig. 2. Cell division and cell wall synthesis genes in the *dcw* cluster in *Escherichia coli* LW1655F$^+$. The presentation is not drawn to scale. All variant genes determined in this study are indicated by arrows and the DNA and protein alterations are given. Asterisks highlight the truncation of the derived gene products of *ftsQ* and *mraY*. Division-specific genes of *dcw* are marked by black boxes, whereas the cell wall synthesis genes are marked by grey boxes. *lpxC*, marked as white box, is involved in lipid A synthesis and is not altered (data not shown). All DNA sequences obtained in this study are deposited at the NCBI under the following accession nos.: AY616586 (the complete *dcw* from *mraZ to lpxC*), AY616658 (16S rRNA gene), AY616615 (*creC*), AY616617 (*ftsK*), AY616613 (*ftsN*), AY616614 (*metB*), AY616590 (*metB*), AY616590 (*minC*), AY616589 (*minD*), AY616588 (*minE*), bankit708954 (*relA* insertion), AY616610 (*spoT*), AY616619 (*sulA*), AY616600 (*ftsB, ygbQ*), AY616602 (*zapA*), AY616597 (*zipA*).

et al., 1990) and with ClustalW (www.clustalw.genome.ad.jp). All DNA sequences obtained in this study are deposited at the NCBI under the following accession nos.: AY616658 (16S rRNA gene), AY616615 (*creC*), AY616586 (all genes encoded within *dcw*), AY616617 (*ftsK*), AY616613 (*ftsN*), AY616614 (*metB*), AY616590 (*metB*), AY616590 (*minC*), AY616589 (*minD*), AY616588 (*minE*), bankit708954 (*relA* insertion), AY616610 (*spoT*), AY616619 (*sulA*), AY616600 (*ftsB, ygbQ*), AY616602 (*zapA*), AY616597 (*zipA*).

Oligonucleotides used for PCR amplification and sequencing

All the oligonucleotides to amplify PCR products and to sequence the *dcw* cluster, full-length genes, and the 16S rRNA gene of the protoplast-type L-form LW1655F$^+$ by primer walking on genomic DNA (f, forward orientation; r, reverse orientation) are listed in the Supplemental Files document, Table S1, Table S2, and Table S3, respectively.

Results and discussion

Confirmation of the *E. coli* K-12 lineage

To confirm the identity of the mutant strain LW1655F$^+$, we determined known genetic markers according to the pedigree of *Escherichia coli* K-12 laboratory strains (Bachmann, 1996) and performed a 16S rRNA gene analysis. We amplified DNA fragments by PCR from the L-form with oligonucleotides deduced from *E. coli* K-12 MG1655F$^-$ genome (Blattner *et al.*, 1997), and then determined their nucleotide sequences. The results are summarized in Table 1, which show that LW1655F$^+$, as outlined in Fig. 1, is an *E. coli* K-12 derivative. This facilitated the screening and comparative analysis of selected L-form genes with the reference genes of *E. coli* K-12 MG1655F$^-$ (Blattner *et al.*, 1997).

Genetic changes in the *dcw* gene cluster of the L-form

To identify genetic differences correlating with an altered division process in the cell wall-less L-form, we sequenced completely the *dcw* region (18.331 bp) from *mraZ* to *lpxC* (Fig. 2; *d*ivision and *c*ell *w*all synthesis gene cluster). *dcw* harbors essential genes for cell division and murein synthesis which are conserved in many distantly related eubacterial genomes (Nanninga, 1998; Tamames *et al.*, 2001). In addition, we cross-examined several division-specific genes distributed along the chromosome (*zapA, zipA, ftsB (ygbQ), ftsK, and ftsN*, and further genes encoding control elements for cell division in *E. coli* (*minCDE*, *sulA*; Margolin, 2003).

In total, we analyzed 35 646 bp of the L-form genome. Altered genes were found exclusively within *dcw* of the L-form affecting the division-specific genes *ftsQ*, *ftsA*, *ftsW, ftsZ*, and the cell wall synthesis genes *mraY* and *murG*. All changes are caused by point mutations. None of them affects known promoters (e.g. *ftsQp2/Qp1*, *ftsA1p, ftsZp4*, *ftsZp3*, *ftsZp2*) retrieved from EcoCyc (Keseler *et al.*, 2005). Figure 2 summarizes the *dcw* genes examined in this study, and shows the genetic changes in the *dcw* locus of the L-form.

Altered *fts* genes of the L-form

The division-specific genes in the L-form *dcw* are affected differently (Fig. 2). A nonsense mutation results in a premature stop codon in *ftsQ*. Missense mutations in *ftsA* and *ftsW* lead to amino-acid (aa) replacements, and a silent mutation is present in *ftsZ*.

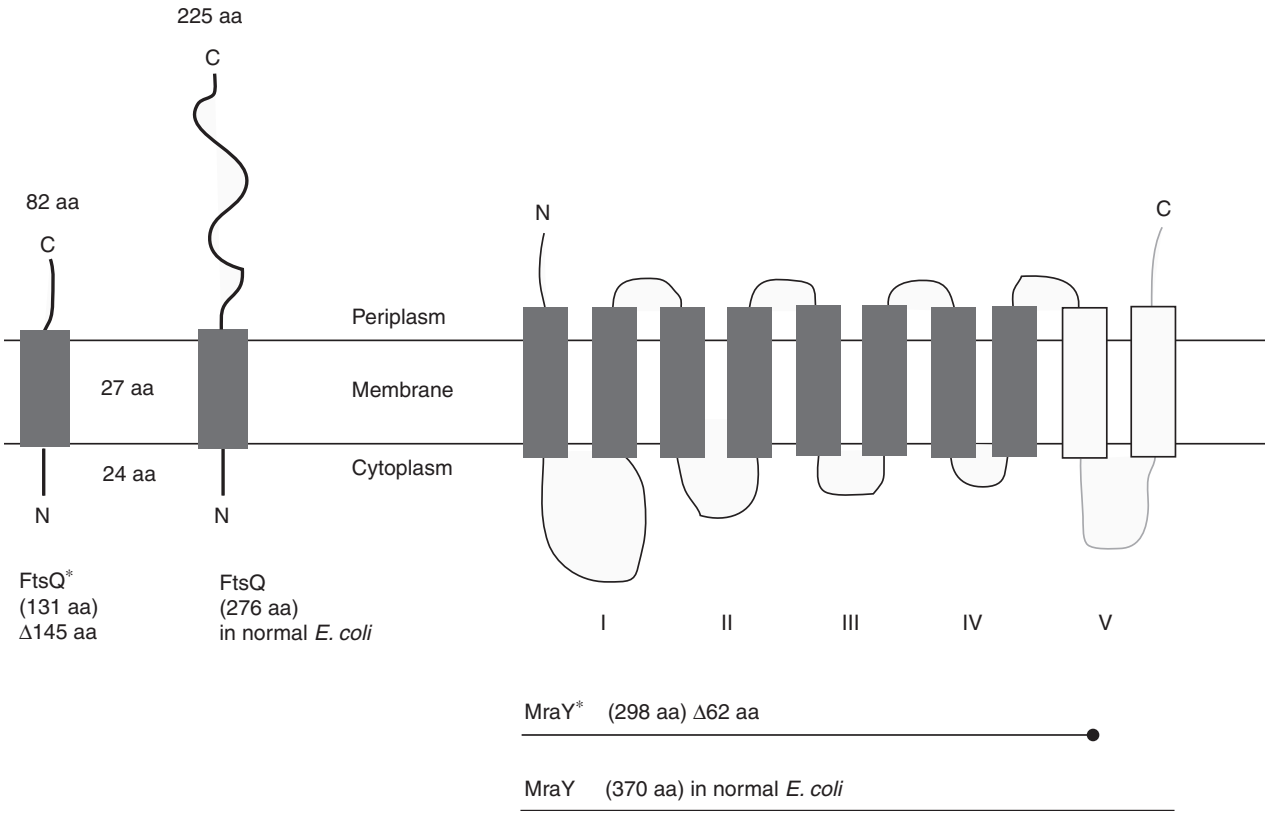

Fig. 3. Topological illustration of the truncated FtsQ* and MraY* in the L-form *Escherichia coli* LW1655F$^+$. FtsQ* of the L-form is marked by an asterisk versus the normal FtsQ. The deletion of the periplasmic loop is indicated. The number of the amino acids spanning the membrane and comprising the cytoplasmic extension are retrieved from Chen *et al*. (2002). MraY* of the L-form is marked by an asterisk versus the normal MraY. The topology of MraY was retrieved from Bouhss *et al*. (1999). Membrane helices are depicted as boxes. Grey boxes represent the helices still present after the truncation. The helices lacking in the L-form are indicated in white. The cytoplasmic loops I–V are indicated.

FtsQ is a bitopic membrane protein (Fig. 3), and previous work from Beckwith and colleagues has shown that its periplasmic extension of 225 aa at the C-terminus is the functionally important domain (Chen *et al*., 2002). It contains residues critical for spatial localization at the Z-ring. Furthermore, it is required to recruit partner proteins for the division process in normal *E. coli*. A deletion of 29 C-terminal aa (13% of the extension removed) still allowed the targeting to the constriction site but the cells were unable to divide, as they could not recruit further division partners (Chen *et al*., 2002). The precise physiological function of FtsQ, like most of the *fts* genes, is unknown. Others have speculated that FtsQ is not only required for cell division but could also play a role in cell wall synthesis during the late phase of cell division (Nanninga, 1998; Chen *et al*., 2002). Recently, FtsQ was shown to harbor a POTRA (*po*lypeptide-*t*ransport-*a*ssociated) domain within its periplasmic extension (aa position 55–126). This domain is discussed to mediate a chaperone function, possibly for the assembly of OM proteins (Sanchez-Pulido *et al*., 2003).

In the L-form, a nonsense mutation (W132TAG) truncates more than half (64%) of this extension from the *ftsQ* product (Fig. 3). Whereas the segment important for recruiting and localization is deleted, the POTRA domain remains preserved. As cell division in *E. coli* requires the correct localization as well as the hierarchical recruitment of division proteins, it is surprising that cell division is not blocked in the L-form. Specifically, the formation of the division subcomplex FtsQLB should be prevented, which has been shown to be conserved among *E. coli*, *Bacillus subtilis* and *Streptococcus pneumoniae* (Buddelmeijer & Beckwith, 2004). As the L-form cells are viable, still can grow, replicate and divide, FtsQ – at least the part lacking from its extension – seems not to be required for division of the wall-less L-form.

Whereas the nonsense mutation suggested a loss-of-function, the missense mutations in the genes *ftsA* and *ftsW* (Figs 2 and 4) currently do not allow conclusions with respect to cell division-specific alterations. The missense in the L-form-FtsA was found at a conserved

Fig. 4. Alignment analysis of FtsA and FtsW from the L-form *Escherichia coli* LW1655F$^+$. The A116E exchange in FtsA is indicated by an arrow. The segment found to play a role in homodimerization is underlined. The D85Y exchange in FtsW is indicated by an arrow. For the alignment analyses the derived primary structures of diverse bacteria were used. The abbreviations of the strains: L, LW1655F$^+$; K12, *E. coli* K-12 MG1655; EcO, *E. coli* O157:H7 EDL933; EcC, *E. coli* CFT073; Shi, *Shigella flexneri* 2a strain 2457T; Vch, *Vibrio cholerae* KIM; Yep, *Yersinia pestis* CO92; Sty, *Salmonella enterica* ssp. *enterica* serovar *Typhi Ty2*; Bap, *Buchnera aphidicola (Baizongia pistaciae)*; Bsu, *Bacillus subtilis* subsp. *Subtilis* strain 168; Psa, *Pseudomonas aeruginosa* (PA01); Nme, *Neisseria meningitidis* strain Z2491; Thm, *Thermotoga maritima* (strain MSB8); Sku, *Spiroplasma kunkelii*.

position in the derived FtsA proteins of diverse bacteria (Fig. 4), e.g. in *Thermothoga maritima*, *B. subtilis*, and even of a *Mycoplasma*-type species (Zhao *et al*., 2004). The A116E exchange resides in subdomain 1c of FtsA, very close to a segment specifically required for homodimerization of the actin-like cytoplasmic protein (Carettoni *et al*., 2003; Rico *et al*., 2004). The gene *ftsW*, encoding an integral membrane protein with unknown function (Boyle *et al*., 1997; Mercer & Weiss, 2002) is changed by a D85Y replacement in the N-terminal part at the membrane-periplasm border (Fig. 4). In contrast to FtsA and FtsW, the primary structure of FtsZ is not altered due to a silent mutation in the L-form (Fig. 2). Also the *minCDE* and *sulA* genes are not changed (data not shown) which play a role in the control for Z-ring formation. Thus, the function of FtsZ (Margolin, 2005) seems to be preserved in the L-form.

L-form cell division has been considered to proceed as in *Mycoplasma* which are naturally occurring cell wall-free bacteria (Margolin, 2000). The question was raised whether proteins other than FtsZ are necessary for L-form cell division (Margolin, 2000). Our study shows that, except for *ftsQ,* all the other division-specific *fts* genes within *dcw*, and also *zapA, zipA, ftsB (ygbQ), ftsK, ftsN, minCDE,* and *sulA* (data not shown) maintain their structure as functional units. Thus, FtsQ is the only division protein not required for L-form division, at the current state of investigation. Current experimental work is aiming at confirming the FtsQ-independent localization of the *fts* gene products at the Z-ring. Preliminary results indicate that L-form cells are not suitable for studying the sufficiency of FtsZ, as a model for division in *Mycoplasma*-like bacteria (C. Hoischen, pers. commun.).

Altered cell wall synthesis genes of the L-form

In addition to the mutations in the *fts* genes, we detected two mutations which affect the cell wall synthesis genes *mraY* and *murG* (Figs 2 and 3). They normally encode enzymes which together catalyze the formation of the lipid-linked disaccharide pentapeptide, lipid II, the precursor required for murein biosynthesis (Nanninga, 1998). The first step of this process involves MraY, an integral membrane enzyme consisting of 10 membrane spanning helices. It catalyzes the binding of UDP-MurNAc-(pentapeptide) to bactoprenol, a membrane-bound C_{55} isoprenoid lipid, to produce lipid I. This is one of the most important steps for murein synthesis in bacteria. In overall terms, it prepares the water-soluble building blocks of the murein sacculus for the transport across the hydrophobic barrier of the CM. Without the MraY function, the murein sacculus cannot be synthesized anymore in the periplasm. Previous mutational analyses have shown that *mraY* is essential for *E. coli*. In *mraY*-deficient mutants, cell division is arrested resulting in lethal phenotypes (Boyle & Donachie, 1998; Bouhss *et al*., 1999). Interestingly to note, MraY was long believed to be a cell division-specific protein, until its physiological function had been elucidated (Nanninga, 1998).

In the L-form, a base deletion causes a frame shift mutation resulting in a premature stop in *mraY*. This prevents the translation of the last two C-terminal helices (Figs 2 and 3) and yields a truncated protein lacking 62 aa

spanning the cytoplasmic loop V (Fig. 3). It suggests that MraY is nonfunctional, or is severely impaired in its function to act together with MurG, an *N*-acetylglucosaminyl transferase. In the L-form, also MurG is altered by a point mutation. The underlying I119V replacement is probably of minor importance, because position 119 in the MurG primary structure is not conserved among enteric bacteria (data not shown). Notably, MraY and MurG have been selected as priority targets for the development of drugs killing murein-containing bacteria (Zawadzke *et al.*, 2003). Our results indicate that L-form cells can grow without the function of MraY, in contrast to normal *E. coli*.

Conclusions

This report represents the first mutation based analysis of a bacterial L-form. It focussed on the genetic background of cell division and cell wall synthesis genes in a bacterial protoplast-type L-form which was derived from *Escherichia coli* (Schuhmann & Taubeneck, 1969). L-form bacteria represent a curiosity in microbiology, and how they divide was open to speculation (Margolin, 2000). Our results suggest that this L-form is able to bypass the function of *ftsQ* and *mraY*, which are absolutely required for cell division and growth in all bacteria with a murein-based cell wall (Nanninga, 1998; Margolin, 2000). Recent experimental studies with *E. coli* indicate that bypass mechanisms and compensatory capacity exist within the bacterial division machinery, that were not anticipated. Studies have shown that subtle changes in the primary structure of cell division proteins can render other division functions dispensable which were believed to be essential [e.g., FtsA can compensate for ZipA (Geissler *et al.*, 2003)]. Moreover, the apparent linearity for recruitment of the components of the division machinery at midcell does not necessarily reflect a temporal order (Goehring & Beckwith, 2005; Goehring *et al.*, 2005). Importantly, the signals used to recruit the division partners seem to be diverse and include protein–protein interactions, regulated assembly of subcomplexes and probably also the recognition of peptidoglycan substrates at the septum (Goehring & Beckwith, 2005; Goehring *et al.*, 2005). In this context and on the basis of our work, we propose that the L-form can be used as a model to further investigate the compensatory capacity and the bypass mechanisms within the bacterial division system. Further experiments are now needed to understand how cell division without concomitant murein sacculus synthesis is accomplished in the L-form. This should be of particular interest to understand whether a pathway exists which enables bacteria to persist without a cell wall, or as cell wall-deficient bacteria, serving as cryptic agents for a variety of human infections. This opinion, and occasional reports on cell wall-free bacteria in medical samples which revert to the walled form upon isolation and cultivation are controversially discussed by microbiologists for more than 50 years (Domingue & Woody, 1997).

As a note of caution concerning the transition of a walled *E. coli* to a protoplast: the mutations determined could reflect the current state of LW1655F$^+$ rather than the primary mutational events leading to the protoplast phenotype. The loss-of-function of *ftsQ* and *mraY* without consequences for viability could represent secondary mutations upon completion of the transition, as the strain has been propagated under laboratory conditions for more than 30 years (Gumpert *et al.*, 2002). Nevertheless, elucidating how this L-form divides will have impact to understand the plasticity of the bacterial cell division and cell wall synthesis machinery in general.

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References

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Bachmann BJ (1996) Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12. *Escherichia coli and Salmonella. Cellular and Molecular Biology*, *Vol. 2*, 2nd edn (Neidhardt FC, Curtiss R III, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M & Umbarger HE, eds), pp. 2460–2488. ASM Press, Washington, DC.

Blattner FR, Plunkett G III, Bloch CA, *et al.* (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**: 1453–1474.

Bouhss A, Mengin-Lecreulx D, Le Beller D & Van Heijenoort J (1999) Topological analysis of the MraY protein catalysing the first membrane step of peptidoglycan synthesis. *Mol Microbiol* **34**: 576–585.

Boyle DS & Donachie WD (1998) *mraY* is an essential gene for cell growth in *Escherichia coli*. *J Bacteriol* **180**: 6429–6432.

Boyle DS, Khattar MM, Addinall SG, Lutkenhaus J & Donachie WD (1997) *ftsW* is an essential cell-division gene in *Escherichia coli*. *Mol Microbiol* **24**: 1263–1273.

Buddelmeijer N & Beckwith J (2004) A complex of the *Escherichia coli* cell division proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region. *Mol Microbiol* **52**: 1315–1327.

Carettoni D, Gomez-Puertas P, Yim L, Mingorance J, Massidda O, Vicente M, Valencia A, Domenici E & Anderluzzi D (2003) Phage-display and correlated mutations identify an essential region of subdomain 1C involved in homodimerization of *Escherichia coli* FtsA. *Proteins* **50**: 192–206.

Chen JC, Minev M & Beckwith J (2002) Analysis of *ftsQ* mutant alleles in *Escherichia coli*: complementation, septal localization, and recruitment of downstream cell division proteins. *J Bacteriol* **184**: 695–705.

Domingue SRGJ & Woody HB (1997) Bacterial persistence and expression of disease. *Clin Microbiol Rev* **10**: 320–344.

Geissler B, Elraheb D & Margolin W (2003) A gain-of-function mutation in *ftsA* bypasses the requirement for the essential cell division gene *zipA* in *Escherichia coli*. *Proc Natl Acad Sci USA* **100**: 4197–4202.

Goehring NW & Beckwith J (2005) Diverse paths to midcell: assembly of the bacterial cell division machinery. *Curr Biol* **15**: R514–526.

Goehring NW, Gueiros-Filho F & Beckwith J (2005) Premature targeting of a cell division protein to midcell allows dissection of divisome assembly in *Escherichia coli*. *Genes Dev* **19**: 127–137.

Gumpert J, Schuhmann E & Taubeneck U (1971) [Ultrastructure of stable L forms of *Escherichia coli* B and W 1655F]. *Z Allg Mikrobiol* **11**: 19–33.

Gumpert J, Hoischen C & Kujau M (2002) Protein expression in L-form bacteria. *Gene Cloning and Expression Technologies* (Weiner MP & Lu Q, eds), pp. 213–226. BioTechniques Press, Eaton Publishing, Westborough, MA.

Keseler IM, Collado-Vides J, Gama-Castro S, Ingraham J, Paley S, Paulsen IT, Peralta- Gil M & Karp PD (2005) EcoCyc: a comprehensive database resource for *Escherichia coli*. *Nucleic Acids Res* **33**: D334–D337.

Margolin W (2000) Themes and variations in prokaryotic cell division. *FEMS Microbiol Rev* **24**: 531–548.

Margolin W (2003) Bacterial division: the fellowship of the ring. *Curr Biol* **13**: R16–R18.

Margolin W (2005) FtsZ and the division of prokaryotic cells and organelles. *Nat Rev Mol Cell Biol* **6**: 862–871.

Mercer KL & Weiss DS (2002) The *Escherichia coli* cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J Bacteriol* **184**: 904–912.

Nanninga N (1998) Morphogenesis of *Escherichia coli*. *Microbiol Mol Biol Rev* **62**: 110–129.

Rico AI, Garcia-Ovalle M, Mingorance J & Vicente M (2004) Role of two essential domains of *Escherichia coli* FtsA in localization and progression of the division ring. *Mol Microbiol* **53**: 1359–1371.

Sanchez-Pulido L, Devos D, Genevrois S, Vicente M & Valencia A (2003) POTRA: a conserved domain in the FtsQ family and a class of beta-barrel outer membrane proteins. *Trends Biochem Sci* **28**: 523–526.

Schuhmann E & Taubeneck U (1969) Stabil L-forms of several *Escherichia coli* strains. *Z Allg Mikrobiol* **9**: 297–313.

Staden R, Beal KF & Bonfield JK (2000) The Staden package, 1998. *Methods Mol Biol* **132**: 115–130.

Tamames J, Gonzalez-Moreno M, Mingorance J, Valencia A & Vicente M (2001) Bringing gene order into bacterial shape. *Trends Genet* **17**: 124–126.

Zawadzke LE, Wu P, Cook L, Fan L, Casperson M, Kishnani M, Calambur D, Hofstead SJ & Padmanabha R (2003) Targeting the MraY and MurG bacterial enzymes for antimicrobial therapeutic intervention. *Anal Biochem* **314**: 243–252.

Zhao Y, Hammond RW, Lee IM, Roe BA, Lin S & Davis RE (2004) Cell division gene cluster in *Spiroplasma kunkelii*: functional characterization of *ftsZ* and the first report of *ftsA* in mollicutes. *DNA Cell Biol* **23**: 127–134.

Supplementary material

The following supplementary material is available for this article online:

Table S1. *dcw*-specific oligonucleotides.

Table S2. Gene-specific oligonucleotides.

Table S3. 16S rRNA-specific oligonucleotides.

This material is available as part of the online article from http://www.blackwell-synergy.com