**Full Paper**

**A requirement for cell elongation protein RodZ and cell division proteins FtsN and DedD to maintain the small rod morphology of *Escherichia coli* at growth temperatures near 8°C**

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As similarly observed in nutrient-poor media at 37°C, *Escherichia coli* forms small rods in nutrient-rich media at temperatures near 8°C, the minimum temperature of growth. A study was initiated to identify proteins required to facilitate the small rod morphology at low temperature. *E. coli* contains three nonessential SPOR domain proteins (DamX, RlpA, and DedD) that have been demonstrated to bind to the septal ring. In contrast to the normal growth and small rod morphology of *damX* and *rlpA* null mutants at 10°C, the *dedD* null mutant exhibited reduced growth and formed filamentous cells. The presence of plasmid-encoded DedD restored growth and small rods. Plasmid-encoded FtsN, an essential SPOR domain protein that functions to stabilize the septal ring and to initiate septation, in the *dedD* null mutant resulted in increased growth and the formation of shorter chained cells. However, plasmid-encoded DedD failed to restore growth and cell division of cells lacking FtsN at 10°C. In contrast to cell division protein DedD, RodZ is a cell elongation protein particularly required for growth at 30°C. However, the *rodZ* null mutant grew similarly as the wild type strain and produced cocci in LB broth at 10°C. Moreover at 10°C, the concerted deletion of *dedD* and *rodZ* resulted in severe inhibition of growth accompanied with the formation of swollen prolate ellipsoids due to a block in septal ring assembly and cell elongation. The data indicate the cellular requirement of both FtsN and DedD for septation as well as RodZ for cell elongation to maintain the small rod morphology at temperatures near 8°C.

In comparison to the growth and small rods of the wild type in M9-glucose minimal media at 37°C, the *dedD* null mutant grew at the same rate and produced elongated cells while the *rodZ* null mutant grew at a slightly slower rate and produced cocci. The data indicate that DedD and RodZ are also required to maintain the small rod morphology in nutrient-poor media, but there is a higher cellular requirement of DedD for growth and cell division in nutrient-rich media at low temperature.

Key Words: cell division; cold adaptation; cold stress; *Escherichia coli*; morphology; small rods; SPOR domain proteins

**Introduction**

Although the optimum growth of *Escherichia coli* is 37°C, balanced growth can be maintained at low temperatures above 8°C. Shifting *E. coli* cells from 37°C to temperatures just above 8°C causes several physiological changes that determine cold adaptation. In addition to transient inhibition of growth and macromolecule synthesis (Shaw and Ingraham, 1967), a shift from 37 to 10°C is characterized by a transient induction of the cold shock response, which includes the induced synthesis of several proteins referred to as cold shock proteins (Jones et al., 1987, 1992). Cold shock proteins comprise several proteins such as ribosomal assembly factor CsdA, whose functions are specifically required for optimal growth at low temperatures (Charollais et al., 2004; Jones et al., 1996). A function of the cold shock response serves to increase...
the level of translatable ribosomes, thereby overcoming the block in translation and promoting growth at low temperatures (Jones and Inouye, 1996).

Morphological changes of rod-shaped E. coli cells also occur at low temperature. Exposing cells to 6°C results in a block in cell division and the formation of long filaments (Shaw, 1968). However, exposing cells to temperatures just above 8°C results in an increase in cell division accompanied with the formation of cocobacilli or small rods (Pierce et al., 2011). CsDA has been demonstrated to be required for the small rod morphology. In contrast to the small rods produced by the wild type strain at 12°C, the csdA null mutant formed elongated cells, indicating that the function of CsDA leads to an increase in septation and the resultant small rod morphology (Pierce et al., 2011). A member of the DEAD-box family of ATP-dependent RNA helicases, CsDA is a 50S ribosomal subunit biogenesis factor and a component of the RNA degradosome at low temperature (Bizebard et al., 2004; Charollais et al., 2004; Prud’homme-Genereux et al., 2004). Although CsDA is not directly involved in cell division, the finding suggested that CsDA links RNA processing and ribosomal assembly with cell division and morphogenesis for cold adaptation (Pierce et al., 2011).

Cellular reproduction of rod-shaped E. coli requires new cell wall synthesis at the lateral wall to accommodate the increase in cell length and at the mid-cell to mediate cell division. Peptidoglycan synthesis at the lateral wall is facilitated by a multiprotein complex that includes penicillin binding protein 2 with actin protein MreB as well as several cell other rod shape maintenance proteins including RodZ (Alyahya et al., 2009; Bendezu et al., 2009, reviewed in Typas et al., 2012; Shiomi et al., 2008). Cells lacking RodZ are round and misshaven as well as exhibit reduced growth particularly at 30°C (Alyahya et al., 2009; Bendezu et al., 2009; Shiomi et al., 2008). Another set of proteins involved in cell division associate with the FtsZ ring that forms at the division site (reviewed in de Boer, 2010; reviewed in Typas et al., 2012). Early cell division proteins, such as FtsA and ZipA, aid in membrane attachment and stabilization of the FtsZ ring. Late cell division proteins, which transform the FtsZ ring to a septal ring competent for septation, are involved in various activities, including chromosome segregation (FtsK) and septal peptidoglycan synthesis (PBP3-FtsW subcomplex). An essential late cell division protein, FtsN functions to stabilize the septal ring and to initiate septation (Gerding et al., 2009; Rico et al., 2010; Ursinus et al., 2004). Binding to the septal peptidoglycan is facilitated by the SPOR domain, which is located at the carboxyl terminus of FtsN (Gerding et al., 2009; Ursinus et al., 2004). E. coli also contains three nonessential SPOR domain proteins (DedD, DamX and RlpA) that also can localize to the septal peptidoglycan (Arends et al., 2010; Gerding et al., 2009).

Effect of nonessential SPOR domain proteins on growth and morphology at high and low temperature

To determine whether the SPOR domain proteins DedD, DamX, and RlpA play a more essential role in cell division at low temperature compared to high temperature, the growth and morphology of dedD, damX, and rlpA null mutants were examined at 37°C and 10°C. At 37°C, the dedD, damX, and rlpA null mutants (Fig. 1A) grew at the same rate as the wild type strain BW25113. At 13°C and 10°C, the damX null and rlpA null mutants grew at a similar rate as the wild type strain (Fig. 1B). In contrast, the dedD null mutant exhibited reduced growth (Fig. 1B).
Cold-sensitive growth of the *dedD* null mutant was also observed on LB agar plates at 13°C (data not shown), further indicating that DedD is essential for growth at low temperature. The wild type and *dedD* null mutant were transformed with vector pBAD33. The *dedD* null mutant was also transformed with pBAD33-*dedD*, which contains *dedD* under control of an arabinose-inducible promoter (Arends et al., 2010). In the presence of 0.2% l-arabinose in the growth media at 10°C, plasmid-encoded DedD complemented the growth of the *dedD* null mutant (Fig. 2). To determine whether increased *ftsN* gene expression could compensate for the absence of DedD at low temperature, the mutant was transformed with pBAD33-*ftsN*, which contains *ftsN* under control of an arabinose-inducible promoter (Arends et al., 2010). In the presence of 0.2% l-arabinose in the growth media, plasmid-encoded FtsN resulted in increased growth of the *dedD* null mutant (Fig. 2).

Microscopic examination of wild type and mutant cells was done to analyze the effect of the SPOR domain proteins on morphology at 37°C and 10°C. At 37°C, the wild type strain BW25113 (Fig. 3A), the *damX* null mutant (Fig. 3B) and the *rlpA* null mutant (Fig. 3C) formed rods. However, the *dedD* null mutant (Fig. 4A) produced fairly elongated cells. In comparison to the rods formed at 37°C (Fig. 3A), the wild type were small rods at 13°C (data not shown) and 10°C (Fig. 3D). Consistent with the normal growth of the mutants at 10°C, the *damX* null mutant (Fig. 3E) and the *rlpA* null mutant (Fig. 3F) also formed small rods. In contrast, the absence of DedD resulted in a pronounced change in morphology. The *dedD* null mutant formed filamentous cells at 10°C (Fig. 4B), ranging in length from 15 μm to 95 μm. As a result of 0.2% l-arabinose in the growth media, plasmid-encoded DedD in the *dedD* null mutant restored the small rod morphology (Fig. 4C). In comparison, plasmid-encoded FtsN resulted in the formation of shorter chained cells (Fig. 4D) but not small rods. In contrast, the presence of 0.2% glucose to repress *dedD* expression from pBAD33-*dedD* or *ftsN* expression from pBAD33-*ftsN* did not result in the formation of small rods or shorter chained cells (data not shown). The data demonstrate that DedD is nonessential for growth at 37°C where the characteristic shape is rod but is required to promote growth and cell division at 10°C where the cells are small rods. Therefore of the three SPOR domain proteins that are nonessential at 37°C, DedD is specifically required to maintain cell division, growth, and small rod morphology at low temperatures. The data also indicate that increased *ftsN* gene expression can partially compensate for the absence of DedD at low temperature.

**Fig. 1.** SPOR Domain protein DedD is specifically required for optimal growth at low temperature.

A. Growth of the wild type strain BW25113, *dedD* null mutant, *rlpA* null mutant, and *damX* null mutant in LB media at 37°C. Following overnight incubation at 37°C in LB media, the cultures were diluted in the same media, and growth was monitored for the times indicated. B. Growth of the wild type strain BW25113, *dedD* null mutant, *rlpA* null mutant, and *damX* null mutant in LB media at 13°C and 10°C. Exponentially growing cells in LB media at 37°C were shifted to 13°C or 10°C. After overnight incubation, the cultures were diluted in LB media and growth was monitored for the times indicated.

**Fig. 2.** Plasmid-encoded DedD or plasmid-encoded FtsN increased the growth of *dedD* null mutant at 10°C.

Growth of BW25113/pBAD33, BW25113Δ*dedD*/pBAD33, BW25113Δ*dedD*Δ*damX*Δ*rlpA*/pBAD33, and BW25113Δ*dedD*Δ*damX*Δ*rlpA*/pBAD33-*ftsN* in LB media in the presence or absence of 0.2% arabinose or 0.2% glucose at 10°C. Exponentially growing cells in LB media at 37°C were shifted to 10°C. After overnight incubation, the cultures were diluted in LB media and growth was monitored for the times indicated.
Effect of cell elongation protein RodZ on growth and morphology at 37°C, 25°C, 13°C, and 10°C

The growth and morphology of the wild type and rodZ null mutant were analyzed to determine the requirement of RodZ to maintain the small rod morphology at low temperature. In comparison to the growth of the wild type strain BW25113 at 37°C and 25°C in LB medium, the growth of the rodZ null mutant was significantly reduced at 25°C compared to 37°C (Fig. 5A), which is consistent with previous observations (Bendezu et al., 2009). At 37°C and 25°C, the wild type strain formed rods (Figs. 6A and 6C). The rodZ null mutant formed round and misshapen cells at 37°C (Fig. 6B) but formed larger round and misshapen cells at 25°C (Fig. 6D), which is consistent with previous observations (Bendezu et al., 2009). However, the rodZ null mutant exhibited different growth and morphological phenotypes in LB broth at lower temperatures. In comparison to the reduced growth observed at 25°C, the rodZ null mutant grew similarly as the wild type strain BW25113 at 13°C and 10°C (Fig. 5B). However on LB agar plates at low temperature, the growth of the rodZ null mutant was severely inhibited (data not shown). The phenotypes of the rodZ null mutant also include non-motility and reduced biofilm formation (Inoue et al., 2007; Niba et al., 2007), which is the probable explanation for the cold-sensitive growth on solid media. Microscopic examination revealed that, in contrast to small rods of the wild type (Fig. 6E), the rodZ null mutant cells were cocci at 10°C (Fig. 6F) and were not the enlarged round and misshapen cells observed at 25°C (Fig. 6D). Therefore, in contrast to cell division protein DedD, cell elongation protein RodZ is required to increase growth at higher temperatures where the shape is rod, but is nonessential for growth at low temperatures in LB broth where the cells are small rods. The data also indicate that RodZ is required for cells to exhibit the small rod morphology at low temperature.

The effect of the concerted absence of DedD and RodZ on growth and morphology at 37°C and 10°C

A dedD rodZ double null mutant was constructed to determine the effect of the concerted absence of DedD and RodZ on growth and morphology at 37°C and 10°C. In comparison to the growth (Fig. 7A) and rod-shaped cells (Figs. 3A and 6A) of the wild type strain BW25113 at 37°C, the dedD rodZ double null mutant displayed reduced growth (Fig. 7A) accompanied with the formation of swollen elongated, misshapen cells (Fig. 8A). At 10°C, the growth of the dedD rodZ double null mutant was drastically inhibited compared to growth of the wild type strain BW25113 (Fig. 7B). In contrast to the small rods of the wild type (Figs. 3D and 6E) formed at 10°C, the dedD rodZ double null mutant produced swollen prolate ellipsoids (Figs. 8B, 8C, and 8D). The data show that the combined absence of DedD and RodZ effects adverse morphological changes at high and low temperature.
Effect of FtsN and a FtsB variant that promotes early cell division on growth and morphology at 10°C

Essential for cell viability and cell division, FtsB is a member of the FtsBLQ subcomplex that recruits FtsW and septal peptidoglycan synthetase FtsI (PBP3) to the septal ring (Gonzalez et al., 2010). A mutation in ftsB, resulting in a replacement of glutamic acid for alanine at position 56, was identified that suppressed the growth and cell di-

![Fig. 4.](image-url) Cellular morphology of dedD null mutant at 37°C and 10°C.
The strains were grown at 37°C or 10°C in LB media and were examined as indicated. For dedD null mutant/pBAD33-dedD and dedD null mutant/pBAD33-ftsN cultures, the growth media contained 0.2% L-arabinose to induce dedD and ftsN expression, respectively. A. dedD null mutant 37°C at OD$_{600}$ of ~0.6. B. dedD null mutant 10°C at the 48 hr. C. dedD null mutant/pBAD33-dedD 10°C at the 48 hr. D. dedD null mutant/pBAD33-ftsN 10°C at the 48 hr. The cells were stained with crystal violet and were viewed under a total magnification of 1,000× (bar = 5 μm).

![Fig. 5.](image-url) Growth of wild type strain BW25113 and the rodZ null mutant at 37°C, 25°C, 13°C, and 10°C.
A. Growth of the wild type strain BW25113 and the rodZ null mutant in LB media at 37°C and 25°C. Following overnight incubation at 37°C or 25°C in LB media, the cultures were diluted in the same media, and growth was monitored for the times indicated. B. Growth of the wild type strain BW25113 and the rodZ null mutant in LB media at 13°C and 10°C. Exponentially growing cultures in LB media at 37°C were shifted to 13°C or 10°C. After overnight incubation, the strains were diluted in LB media, and growth was monitored for the times indicated.
vision requirement for SPOR domain protein FtsN (Liu et al., 2015) at 30°C. The FtsN suppressing mutation in ftsB results in premature initiation of septal peptidoglycan synthesis and cell constriction, bypassing the stimulatory effect of FtsN normally required for these activities (Liu et al., 2015). Because the ftsB mutation suppresses the requirement for FtsN and because the ftsB mutation results in a preferential shift towards septal peptidoglycan synthesis and septation, the effect of low temperature on growth and morphology on wild type strain TB28, strain BL167 [ftsBES6A], and strain BL173 [ftsBES6A ΔftsN] was analyzed at 10°C. As observed with the rodZ null mutant...
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Fig. 8. Cellular morphology of dedD rodZ double null mutant at 37°C and 10°C. The dedD rodZ double null mutant was grown at 37°C or 10°C in LB media and was examined as indicated. A. dedD rodZ double null mutant 37°C at OD_{420} of ~0.6. B. dedD rodZ double null mutant 10°C at the 48 hr. C. dedD rodZ double null mutant 10°C at the 72 hr. D. dedD rodZ double null mutant 10°C at the 120 hr. The cells were stained with crystal violet and were viewed under a total magnification of 1,000x (bar = 5 μm).

Effect of DedD and RodZ on growth and small rod morphology in nutrient-poor media at 37°C

Because the morphology of E. coli wild type cells grown in nutrient-poor media is small rods, the effect of DedD and RodZ on growth and morphology of cells cultured in M9-glucose minimal media at 37°C was examined. As shown in Fig. 10A, the dedD null mutant grew at the same rate as the wild type, and the rodZ null mutant formed coccus-shaped cells (data not shown). However, in the presence of 0.2% L-arabinose in the growth media, plasmid-encoded FtsN resulted in increased growth (Fig. 10B) and the formation of coccus-shaped cells (data not shown), as similarly observed as strain BL167 [ftsB^{E56A} ΔftsN] (Figs. 9A and 9D) at 10°C. Therefore, the ftsB mutation cannot suppress the requirement of FtsN for growth and cell division at 10°C. Strain BL73 [ftsB^{E56A} ΔftsN] was transformed with vector pBAD33 or pBAD33-ftsN containing ftsN under control of an arabinose-inducible promoter (Arends et al., 2010). As observed with strain BL167 [ftsB^{E56A} ΔftsN], strain BL73 with vector pBAD33 had reduced growth (Fig. 9B) and formed filamenous cells (data not shown). However, in the presence of 0.2% L-arabinose in the growth media, plasmid-encoded FtsN resulted in increased growth (Fig. 9B) and the formation of coccus-shaped cells (data not shown), which was also transformed with pBAD33-dedD, which contains dedD under control of an arabinose-inducible promoter (Arends et al., 2010). In the presence of 0.2% L-arabinose in the growth media, plasmid-encoded DedD exacerbated the growth defect (Fig. 9B) and failed to restore the coccus morphology (data not shown) at 10°C. Although increased ftsN gene expression can partially compensate for the absence of DedD at low temperature, the data indicate that increased dedD gene expression cannot compensate for the absence of FtsN for growth and cell division at 10°C.
Exponentially growing cultures in LB media at 37°C were shifted to 10°C. After overnight incubation, the strains were diluted in LB media, and growth was monitored at 10°C for the times indicated. A. Growth of wild type strain TB28, strain BL167 [ftsB\textit{E56A}], and strain BL173 [ftsB\textit{E56A} \text{ΔftsN}] at 10°C. B. Growth of the BL173 [ftsB\textit{E56A} \text{ΔftsN}]/pBAD33, BL173 [ftsB\textit{E56A} \text{ΔftsN}]/pBAD33-\text{ftsN}, and BL173 [ftsB\textit{E56A} \text{ΔftsN}]/pBAD33-\text{dedD} in LB media at 10°C. For BL173 [ftsB\textit{E56A} \text{ΔftsN}]/pBAD33-\text{ftsN} and BL173 [ftsB\textit{E56A} \text{ΔftsN}]/pBAD33-\text{dedD} cultures, the growth media contained 0.2% L-arabinose to induce \text{ftsN} and \text{dedD} expression. C. TB28 at the 48 hr. D. BL167 [ftsB\textit{E56A}] at the 48 hr. E. BL173 [ftsB\textit{E56A} \text{ΔftsN}] at the 48 hr. The strains were grown at 10°C in LB media and were examined as indicated. The cells were stained with crystal violet and were viewed under a total magnification of 1,000× (bar = 5 μm).

**Discussion**

FtsN, the prototype \textit{E. coli} SPOR domain protein, is required for growth and cell division at low temperature. A FtsB variant that causes premature initiation of septal peptidoglycan synthesis and septation (Liu et al., 2015) could not suppress the growth and morphological requirement for FtsN at 10°C. Although strain BL167 [ftsB\textit{E56A}] grew like the wild type and produced coccus-shaped cells, the growth of strain BL173 [ftsB\textit{E56A} \text{ΔftsN}] was drastically inhibited and the mutant cells were filamentous at 10°C. The presence of plasmid-encoded FtsN in strain BL173 [ftsB\textit{E56A} \text{ΔftsN}] restored growth and the coccus morphology. Therefore, even in the presence of a FtsB variant that promotes early cell division, the data indicate that FtsN is absolutely required for the preferential shift towards septation at 10°C. SPOR domain proteins DamX and RlpA are not required for growth and cell division at 10°C. In contrast, SPOR domain protein DedD has a major role in cell division at low temperature as demonstrated by the formation of small rods at low temperature. The finding that plasmid-encoded FtsN increased the growth of the \text{dedD} null mutant but resulted in the appearance of shorter chained cells, but not small rods, demonstrates that FtsN and DedD have distinct roles in cell division at low temperature. This is also supported by the finding that plasmid-encoded-DedD could not restore growth and cell division to cells lacking FtsN at 10°C.

Cell elongation protein RodZ is also required for the maintenance of the small rod shape at low temperature. At 10°C, cells lacking RodZ were cocci and were not the large round, misshapen cells observed at 25°C. Furthermore, in contrast to the reduced growth observed at 25°C, the \text{rodZ} null mutant grew similarly as the wild type strain at 13°C and 10°C in LB broth, implying that the metabolism rate of the small rods of wild type is comparable to the metabolism rate of the coccoid cells of the \text{rodZ} null mutant at low temperature. This is also consistent with the finding that the coccus-shaped cells of strain BL167 [ftsB\textit{E56A}] grew similarly as the small rods of the wild type at 10°C. Small rods and coccoid cells are produced by wild type \textit{E. coli} under certain growth limiting conditions (Lange and Hengge-Aronis, 1991; Signoretto et al., 2002;
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Young, 2007). Selective pressure, such as nutrient limitation, elicits a decrease in cell size as a result of an increase in cell division (Marshall et al., 2012; Young, 2007). Small cells have a high surface to volume ratio, which allows for optimal influx of nutrients and efflux of wastes. Furthermore, the morphological alteration of a rod-shaped cell to a smaller coccoid cell conserves energy during nutrient shortage (Young, 2007). Therefore, the rod to small rod transformation observed in wild type *E. coli* is an adaptive response to slow growth conditions at temperatures near 8°C. This is supported by the cellular requirement for DedD to increase cell division resulting in small rods and growth at low temperature. Furthermore, the severe growth inhibition and swollen prolate ellipsoids of the *dedD rodZ* double null mutant compared to normal growth and elongated cells of the *dedD* null mutant in LB media at 37°C indicate that there is a higher cellular requirement of DedD for cell division to maintain growth and the small rod morphology in nutrient-rich media at low temperature.

The physiological roles of DedD and RodZ to effect shape were further evident by the phenotypes displayed by *dedD rodZ* double null mutant at high and low temperature. At 37°C, the *dedD rodZ* double null mutant cells formed swollen elongated, missshapen cells. However at 10°C, the *dedD rodZ* double null mutant formed swollen ellipsoids. The other known reported *E. coli* mutant to form swollen prolate ellipsoids is the *rodA ftsZ* temperature-sensitive (Ts) double mutant at 42°C (Begg and Donachie, 1985). The Ts mutation in *rodA* resulted in the formation of cocci due to a block in cell elongation, while the *ftsZ* mutation caused filaments due to a block in septal ring assembly (Begg and Donachie, 1985; Taschner et al., 1988). This swollen morphology is due to new peptidoglycan synthesis in the absence of cell elongation and septal ring formation (Begg and Donachie, 1985). The formation of the swollen prolate ellipsoids by *dedD rodZ* double null mutant at 10°C indicates that DedD is required for septal ring assembly and RodZ is required for cell elongation for cells to exhibit the small rod morphology at low temperature.
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References

Aliyahya, S. A., Alexander, R., Costa, T., Henriques, A. O., Emonet, T. et al. (2009) RodZ, a component of the bacterial core morphogenic apparatus. Proc. Natl. Acad. Sci. USA, 106, 1239–1244.

Arends, S. J. R., Williams, K., Scott, R. J., Rolong, S., Popham, D. L. et al. (2010) Discovery and characterization of three new Escherichia coli septal ring proteins that contain a SPOR domain: DamX, DedD, and RlpA. J. Bacteriol., 192, 242–255.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y. et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol., 2, 1–11.

Begg, K. J. and Donachie, W. D. (1985) Cell shape and division in Escherichia coli: experiments with shape and division mutants. J. Bacteriol., 163, 615–622.

Bendeuz, F. O., Hale, C. A., Bernhardt, T. G., and de Boer, P. A. J. (2009) RodZ (YfGA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in E. coli. The EMBO J., 28, 193–204.

Bizebard, T., Ferlenghi, I., Iost, I., and Dreyfus, M. (2004) Studies on three Escherichia coli DEAD-box helicases point to an unwinding mechanism different from that of model DNA helicases. Biochemistry, 43, 7857–7866.

Charollais, J., Dreyfus, M., and Iost, I. (2004) CsdA, a cold-shock RNA helicase from Escherichia coli, is involved in the biogenesis of 50S ribosomal subunit. Nucleic Acids Res., 32, 2751–2759.

Cherepanov, P. P. and Wackernagel, W. (1995) Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene, 158, 9–14.

de Boer, P. A. J. (2010) Advances in understanding E. coli cell fission. Curr. Opin. Microbiol., 13, 730–737.

Gerding, M. A., Liu, B., Beneditu, F. O., Hale, C. A., Bernhardt, T. G. et al. (2009) Self-enhanced accumulation of FtsS at division sites and roles for other proteins with a SPOR domain (DamX, DedD, and RlpA) in Escherichia coli cell constriction. J. Bacteriol., 191, 7383–7401.

Gonzalez, M. D., Akbay, E. A., Boyd, D., and Beckwith, J. (2010) Multiple interactions domains in FtsL, a protein component of the widely conserved FtsLBQ cell division complex. J. Bacteriol., 192, 2757–2768.

Inoue, T., Shingaki, R., Hirose, S., Waki, K., Mori, H. et al. (2007) Genome-wide screening of genes required for swarming motility in Escherichia coli K-12. J. Bacteriol., 189, 950–957.

Jones, P. G. and Inouye, M. (1996) RfbA, a 30S ribosomal binding factor, is a cold-shock protein whose absence triggers the cold-shock response. Mol. Microbiol., 21, 1207–1218.

Jones, P. G., VanBoglen, R. A., and Neidhardt, F. C. (1987) Induction of proteins in response to low temperature in Escherichia coli. J. Bacteriol., 169, 2092–2095.

Jones, P. G., Cashel, M., Glaser, G., and Neidhardt, F. C. (1992) Function of a relaxed-like state following temperature downshifts in Escherichia coli. J. Bacteriol., 174, 3993–3914.

Lange, R. and Hengge-Aronis, R. (1991) Growth phase-regulated expression of bola and morphology of stationary-phase Escherichia coli cells are controlled by the novel sigma factor σ27. J. Bacteriol., 173, 4474–4481.

Shaw, M. K. (1968) Formation of filaments and synthesis of macromolecules at temperatures below the minimum for growth of Escherichia coli. J. Bacteriol., 95, 221–230.

Shaw, M. K. and Ingraham, J. L. (1967) Synthesis of macromolecules by Escherichia coli near the minimal temperature for growth. J. Bacteriol., 94, 157–164.

Shibmo, D., and Niki, H. (2013) A mutation in the promoter region of zipA, a component of the divisome, suppresses the shape defect of RodZ-deficient cells. MicrobiologyOpen, 2, 798–810.

Shibmo, D., Sakai, M., and Niki, H. (2008) Determination of bacterial rod shape by a novel cytoskeleton membrane protein. The EMBO J., 27, 3081–3091.

Signoretto, C., Lleo, M. M., and Canepari, P. (2002) Modification of peptidoglycan synthesis to bacterial growth and cell division regulation of peptidoglycan synthesis to bacterial growth and morphology. Nat. Rev. Microbiol., 10, 123–136.

Ursinus, A., van den Ent, F., Brechtel, S., de Pedro, M., Holtje, J.-V. et al. (2004) Murein (peptidoglycan) binding property of the essential cell division protein FtsN from Escherichia coli. J. Bacteriol., 186, 6728–6737.

Young, K. D. (2007) Bacterial morphology: why have different shapes? Curr. Opin. Microbiol., 10, 596–600.