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Identification of a novel function for the FtsL cell division protein from *Escherichia coli* K12

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ABSTRACT

Analysis of the essential cell division protein FtsL demonstrates the partial conservation of a cysteine-pair within the trans-membrane region which itself is flanked by histidine-pairs in the cytosol and periplasm. Similar arrangements of such amino acids are seen in proteins known to transport/bind metal ions in biological systems. Heterologous expression of *ftsL* in *Escherichia coli* K12 confers a Zn(II)-sensitive phenotype and alteration of the candidate metal-ion binding residues cysteine or histidine substantially alters this phenotype. Whilst the cysteine/histidine replacement derivatives of *ftsL* were able to complement an otherwise *ftsL*-null strain, the derivative carrying *ftsL* lacking the cysteine pair was sensitive to raised metal-ion concentrations in the media. We show that *ftsL* can confer a metal-ion sensitive phenotype and that trans-membrane cysteine residues play a role in FtsL function in elevated metal-ion concentrations.

1. Introduction

During cell division, a complex of proteins (the divisome) forms at the nascent cell septum that drives the processes that result in cell separation, many of the component proteins are essential to this progress, one of which is FtsL [1,2]. The FtsL protein from *Escherichia coli* (*E. coli*) K12 is a small (approx. 13 kDa) cytoplasmic membrane protein that has a small cytoplasmic amino-terminal domain (37 amino acids), which is linked to a larger carboxyl periplasmic domain (64 amino acids) by a single membrane-spanning segment (20 amino acids) [3,4] (Fig. 2B). This protein is both essential and integral to the process of cell division [3] with cellular FtsL depletion resulting in the formation of filamentous cells. This depletion can also result in the production of Y-shaped filaments with one or more cell extensions [3], or the production of filaments with bulges or lysis bubbles at septal sites [3–6] indicating that a reduction in FtsL abundance affects the cell envelope structure. FtsL is a bitopic membrane protein with three essential functional domains [3]. FtsL has been shown to have three properties that have been mapped to four structural regions [3,7,8]. These features appear to be conserved at either the primary sequence or structural level throughout prokaryotic FtsL homologues [3,8,9]. The role of FtsL is thought to be structural and it is involved in a range of interactions within the divisome, in particular with the FtsB and FtsQ proteins whose structure and topology is similar to that of FtsL [9].

In this study we analysed the contribution of partially conserved amino acid residues whose distribution within the protein were suggestive of a role in metal-ion transport. Our findings show that heterologous expression of FtsL from a weak promoter confers Zn(II)-sensitivity on the host cell and this is consistent with a model in which a sub-set of FtsL homologues, restricted to the enterobacteriaceae, may be capable of trafficking Zn(II) and perhaps other metal-ions. Although this is not an essential role in *E. coli* K12, derivatives lacking these residues were sensitive to elevated metal-ion concentrations in the media suggesting that this distinct sequence may represent an adaptation to metal-ion exposure in the environment.

2. Materials and methods

2.1. Bacterial strains and plasmids

The bacterial strains used for this study were, JM109 (*recA*1, *supE*44, *endA*1, *hsdR*17, *gyrA*96, *relA*1, *thi*, Δ(*lac-proAB*)) [10], KS272 (*F*⁻, Δ*lacX*74, *gal*E, *gal*K, *thi*, *rps*L, Δ*pho*A (PvuII)) [11], TOP10F′ (F′ {*lacIq tetR*} *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*Δ*M15* Δ*lacX74 deoR recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL endA1 nupG*) (Invitrogen), RW3110 (Δ*zntA*) [12], JMG15 (J. Beckwith, Harvard Medical School).

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The plasmids used were: pBAD18, pBAD18 (Cm) [13], pBAD*ftsL* [3] and their derivatives described in the text. All strains were cultured in Luria broth unless otherwise stated, and antibiotics (Melford Laboratories) were added to media at standard concentrations [14].

2.2. Chemicals, reagents and laboratory consumables

General laboratory chemicals were obtained from Sigma–Aldrich or Fisher Scientific UK and were of analytical grade or higher. Other reagent and consumable suppliers are listed in the text were appropriate.

2.3. Manipulation of DNA molecules

DNA manipulations were carried out in accordance with Sambrook et al. [14] unless otherwise specified.

2.4. Disk assays

A cell suspension (in sterile iso-saline) was spread across the surface of the medium and the plate was allowed to dry. Filter paper disks impregnated with 10 μl of 1 M $ZnSO_4$ were placed onto each plate and the bacterial lawn was incubated at 37 °C overnight. Measurements of the zone of bacterial growth inhibition were taken the following day.

2.5. Bacterial growth studies carried out in batch culture

E. coli strains were grown in liquid culture (5 ml) to an optical attenuance (D_{590}) of 0.4 and subcultured into fresh LB medium. The resultant cultures were used to inoculate (1% v/v) fresh media (25 ml) and growth was continued in shake-culture at 37 °C. The optical attenuance (D_{590}) of these cell cultures was periodically monitored.

2.6. Site directed mutagenesis

Site directed mutagenesis was carried out using the Stratagene QuikChange™ Site Directed Mutagenesis kit, in accordance with the manufacturer's instructions using the oligonucleotides, $H_{19}A$, $H_{22}A$-sense: 5′-GATGGGAAGCGCCGAGCGCGCTGCATTGCCTGGTG-3′;$H_{19}A$,$H_{22}A$-antisense: 5′-CACCAGGCAATGCAGCGCGCTCGGCGCT TCCCATC-3′; $C_{41}SC_{45}S$-sense: 5′-CTGCCACTCTCCCTGTTCATTTCCAT-TA TTTTGAC-3′; $C_{41}SC_{45}S$-anti-sense: 5′-GTCAAAATAATGGAAAT-GAACAGGGAGAG TGGCAG-3′; $H_{58}A$,$H_{59}A$-sense: 5′-AACCACGGCG GCCGCTACCCGTTTACTGACCGC-3′ and $H_{58}A$,$H_{59}A$-anti-sense: 5′-G CGGT CAGTAAACGGGTAGCGGCCGCCGTGGTT-3′. Oligonucleotides used for site-directed mutagenesis were synthesised by Invitrogen. The nucleotides sequences of all resultant constructs were confirmed using an ABI Prism BigDye Terminator v3.0 Cycle Sequencing Ready Reaction kit (supplied by Perkin–Elmer Life Science Products). Electrophoresis and visualisation of the extension products were accomplished using an ABI Prism 373 DNA sequencer.

2.7. Strain construction by generalised transduction

The *ftsL*::Tn*phoA* L81ΔIS*50*R-disrupted gene was transferred to recipient strains by generalised transduction with P1 bacteriophage [15], with selection for kanamycin resistance. P1 bacteriophage was obtained from NCIMB; Cat. No. #11291.

2.8. Complementation of KS272ΔftsL::TnphoA with various ftsL constructs

E. coli (KS272Δ*ftsL*::Tn*phoA*) containing either pBAD*ftsL* (Cm), pBAD*ftsL* $H_{19}H_{22}$ (Cm), pBAD*ftsL* $C_{41}C_{45}$ (Cm) or pBAD*ftsL* $H_{58}H_{59}$ (Cm) were inoculated from plate colonies into LB medium that contained chloramphenicol, arabinose (0.2% w/v). The *ftsL* derivatives were excised from pBAD18 with the restriction enzymes *Hind*III and *Nhe*I and were introduced into the corresponding sites of pBAD18 (Cm). All plasmid constructs were verified by PCR with specific and vector-based primers, and were confirmed by restriction analysis. Cultures were grown in shake culture overnight at 37 °C. The resulting cultures were diluted (1% v/v) in triplicate into chloramphenicol containing LB media that was also supplemented with arabinose (0.2% w/v) or arabinose plus Zn(II) (0.5 mM). The initial optical attenuance (D_{590}) of these cultures was taken and growth was continued overnight in shake culture. The following morning the attenuance of each culture was taken again and the ability of the FtsL derivatives to complement the chromosomal *ftsL*::Tn*phoA* disruption was given as a percentage of the D_{590} achieved by the control *E. coli* (KS272Δ*ftsL*::Tn*phoA*):pBAD*ftsL* (Cm) strain.

3. Results

3.1. The topology of FtsL from Escherichia coli K12 is consistent with that of a metal-ion transport protein

The FtsL primary polypeptide sequence contains six histidine residues (four of which lie immediately either side of the transmembrane region) and two cysteine residues (within the transmembrane region) that are extensively or partially conserved throughout FtsL homologues (Fig. 1).

The hydrophobic region, known to be a trans-membrane helix [3], can be modelled using a "helical wheel" projection (Fig. 2A) and this clearly shows that the cysteine residues are closely spaced on the hydrophilic face of the helix and the opposing face is hydrophobic. A similar plot for the mercuric-ion transport protein MerC [16] is shown for comparison. The amphipathic nature and localisation of the sulphur bearing residues is very similar to known metal-ion transport proteins (Fig. 2B).

3.2. FtsL production alters the ability of E. coli cells to tolerate Zn(II)

ftsL from *E. coli* K12 was expressed from a heterologous promoter within pBAD18 (host RW3110Δ*zntA*). The focus on Zn(II) was due to experimental evidence linking Zn(II)-depletion with the arrest of cell division in *E. coli* K12 [17], thus a host cell line lacking a gene (*zntA*) encoding a Zn(II)-exporter (RW3110Δ*zntA*) was used to maximise the sensitivity of these assays. Cells containing either pBAD18 or pBAD *ftsL* were assayed for Zn(II)-tolerance assays using disk assays. Both strains cultured on LB agar displayed equivalent levels of tolerance to Zn(II), however, the presence of *ftsL* conferred a comparative decrease in Zn(II)-tolerance (Table 1).

When grown in LB medium supplemented with arabinose (0.5% w/v) both strains showed similar growth characteristics (Fig. 3A). Growth of control strain (pBAD18) in Zn(II)-supplemented media resulted in an increase in lag phase (Fig. 3B–D). In contrast, strains carrying pBAD:*ftsL* grown with arabinose and Zn(II) (Fig. 3B–D), a Zn(II)-dependent increase in lag phase and a decrease in stationary phase growth were observed in comparison with control cells.

3.3. Cysteine/histidine replacement in FtsL alters the Zn(II)-sensitive phenotype

Site-directed mutagenesis was used to create derivatives of *ftsL* directing $H_{19}A$,$H_{22}A$ $C_{41}S$,$C_{45}S$ and $H_{58}A$,$H_{59}A$ replacements and their stable expression was confirmed (data not shown). *E. coli* strains carrying these derivatives showed almost identical similar growth characteristics to w.t. when cultured in LB medium (Fig. 4A), demonstrating that none of these derivatives encodes an intrinsically deleterious product.

```
                                        *   *
E.coli K12              MISRVTEALSKVKGSMGSHERHALPGVIGDDLLRFGKLPL      40
E.coli O157             MISRVTEALSKVKGSMGSHERHALPGVIGDDLLRFGKLPL      40
K.pneumoniae            MIGRVTEALSKVKGSIGSNERHALPGVIGDDLLRFGKLPL      40
C.koseri                MISRVTEALSKVKGSTGITNRHALPGVIGDDLLRFGKLPL      40
Y.ruckeri               ----------------------MVGVIGADLIRNAKIPL      17
E.amylovora             MIG---------------NERHSLPGVIGGDLIRHGKIPV      25

Consensus               Misrvtealskvkgs-gs-erhalpGVIGdDLlRfgKlPl

                        *   *            **
E.coli K12              CLFICIILTAVTVVTTAHHTRLLTAQREQLVLERDALDIE      80
E.coli O157             CLFICIILTAVTVVTTAHHTRLLTAQREQLVLERDALDIE      80
K.pneumoniae            CLFICIIITAITVVTTAHHTRLLTAQREQLVLERDALDIE      80
C.koseri                CLFICIILTAVTVVTTAHHTRLLTAQREQLVLERDALDIE      80
Y.ruckeri               ILLVAVLVSAVLVVTTAHRTRLLTAEREQLVLERDALDIE      57
E.amylovora             VLFIAVLISALFVVTTAHKTRLLTAQREQLVLERDALDIE      65

Consensus               cLficii-tAvtVVTTAHhTRLLTAqREQLVLERDALDIE


E.coli K12              WRNLILEENALGDHSRVERIATEKLQMQHVDPSQENIVVQK     121

E.coli O157             WRNLILEENALGDHSRVERIATEKLQMQHVDPSQENIVVQK     121
K.pneumoniae            WRNLILEENALGDHSRVERIATEKLQMQHVDPSQENIVVQK     121
C.koseri                WRNLILEENALGDHSRVERIATEKLQMQHVDPSQENIVVQK     121
Y.ruckeri               WRNLILEENALGDHSRVERIATEKLQMQHVDPAQENIVVKQ      98
E.amylovora             WRNLILEENALGDHSRVERIATEKIQMQHVDPSQENIVVHN     106

Consensus               WRNLILEENALGDHSRVERIATEKlQMQHVDPsQENIVVqk
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Fig. 1. Sequence alignment of FtsL primary sequences from a range of organisms with decreasing homology to *E. coli* FtsL from top to bottom.

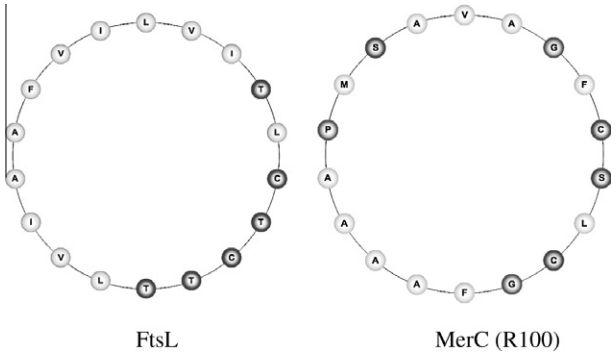

Fig. 2A. Helical wheel representation of the trans-membrane region of *E. coli* K12 FtsL and the mercuric ion transport protein, MerC from the *mer*-operon of plasmid R100. Hydrophilic residues (light grey) and hydrophobic residues (dark grey) are shown.

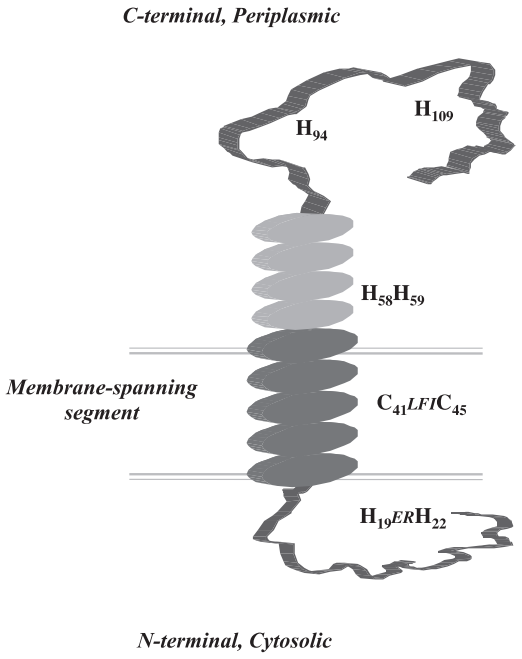

Fig. 2B. Graphic representation of FtsL topology showing the position of cysteine and histidine residues. The leucine zipper region (light grey) and the trans-membrane region (dark grey) are labelled.

In Zn(II)-supplemented medium (0.5 mM) (Fig. 4B), a clear difference can be seen between the strains carrying pBAD*ftsL* cells (open square) and the pBAD18 control (open circles), with the pBAD*ftsL* conferring Zn(II)-sensitivity on the host cell. The derivative pBAD*ftsL* $H_{19}H_{22}$ (closed circles) conferred a very similar growth pattern to pBAD*ftsL* showing that, under these conditions, it is similar to w.t. *ftsL*.

The derivatives pBAD*ftsL* $C_{41}C_{45}$ (closed squares) and pBAD*ftsL* $H_{58}H_{59}$ (closed triangles) conferred intermediate Zn(II)-sensitivity phenotypes between the pBAD18 negative control and the pBAD*ftsL* positive control (Fig. 4B), implicating these residues in the generation of metal-ion sensitivity under these conditions.

3.4. The histidines (19, 22, 58, 59) and the cysteines (41,45) of the FtsL primary sequence are not essential for cell division

The derivatives of *ftsL* were tested for their ability to complement a chromosomal *ftsL*::Tn*phoA* null mutation. The *ftsL* derivatives were introduced into the vector pBAD18 (Cm) and the chromosomal *ftsL*::Tn*phoA* null mutation was introduced into the genome of cells using generalised P1 transduction with lysates prepared from *E. coli* (JMG15Δ*ftsL*::Tn*phoA*). The results (Table 2) showed that all of the *ftsL* derivatives were functional although the $C_{41}S$,$C_{45}S$ derivative did not complement to the same degree as w.t. *ftsL*. In the presence

Table 1
The effect of FtsL on *E. coli* (RW3110Δ*zntA*) Zn(II)-ion tolerance.

Plasmid contained in cell	Apparent Zn(II)-tolerance (% of control strain)	
	LB medium	LB medium + arabinose
pBAD18	100 ± 4	100 ± 5
pBAD*ftsL*	100 ± 5	83 ± 4

Disk assays of cultures grown in LB medium, or media supplemented with either arabinose (0.5% w/v) were carried out on matching LB agar plates. Growth was monitored after incubation for 24 h at 37 °C. The data presented are the mean average diameters of the zones of growth inhibition that surround each disk. These data are shown as an inverse percentage of the mean clearing zone diameters obtained for control pBAD18 containing cells, which were given an arbitrary 100% value. Standard deviations are shown ($n = 3$).

of 0.5 mM Zn(II) all of the *ftsL*-derivatives showed a diminished level of complementation in comparison with w.t., however, the $C_{41}S$,$C_{45}S$ derivative was most strikingly sensitive to Zn(II)-supplementation (Table 2). The sensitivity of these strains to a range of metal-ions was tested including Ni(II), Cu(II), Cd(II) and Co(II), of these only Co(II) showed an effect and only with the $C_{41}S$,$C_{45}S$ derivative of FtsL (data not shown).

4. Discussion

The process of cell division is carried out by the co-ordinated action of many proteins that make up the septal machinery and FtsL lies at the centre of this process [1,2].

Database analysis shows that the cysteines residues lying at positions 41 and 45 in the *E. coli* K12 FtsL primary sequence are conserved but only in a small subset of related strains including *Salmonella*, *Citrobacter* and *Enterobacter* species. Conversely, the histidine residues addressed in this study (position 19, 22, 58 and 59) are relatively well conserved throughout a wide range of FtsL homologues. Modelling of the *E. coli* K12 FtsL secondary structure demonstrated that these cysteine and histidine residues, known to be associated with the coordination of metal ions, were spatially organised in a similar fashion to those in known metal-permease system forming putative sites for metal-ion binding or a pathway for their transfer across the cytoplasmic membrane.

The $C_{41}LFIC_{45}$ motif of *E. coli* K12 FtsL is similar to that seen in a many metal-ion binding proteins throughout biology e.g. the heavy-metal-associated motif (GxxCxxC) [18]. In addition, its location within the predicted hydrophilic surface of an amphipathic membrane-spanning helix is similar to known metal-permeases e.g. MerC (Fig. 2B) and the soft metal-ion translocating P-type ATPases [19–22]. The relative orientation of these cysteine residues allows for them to be very closely spaced when present in a helical secondary structure (Fig. 2B) and thus forming a site capable of the transfer/chelation of metal-ions.

We hypothesise that, since FtsL has been shown to form dimeric and multimeric complexes [8], it is possible that two or more of these membrane-spanning segments may join together to form a homo or hetero-multimer that has multiple cysteine residues situated at its centre. Hetero-multimeric complexes that include FtsL are formed with other bitopic septal-ring proteins and it is interesting to note that one of these, *E. coli* K12 FtsI [4], also contains two cysteine residues in a CgC motif that lies in the transmembrane region of the protein.

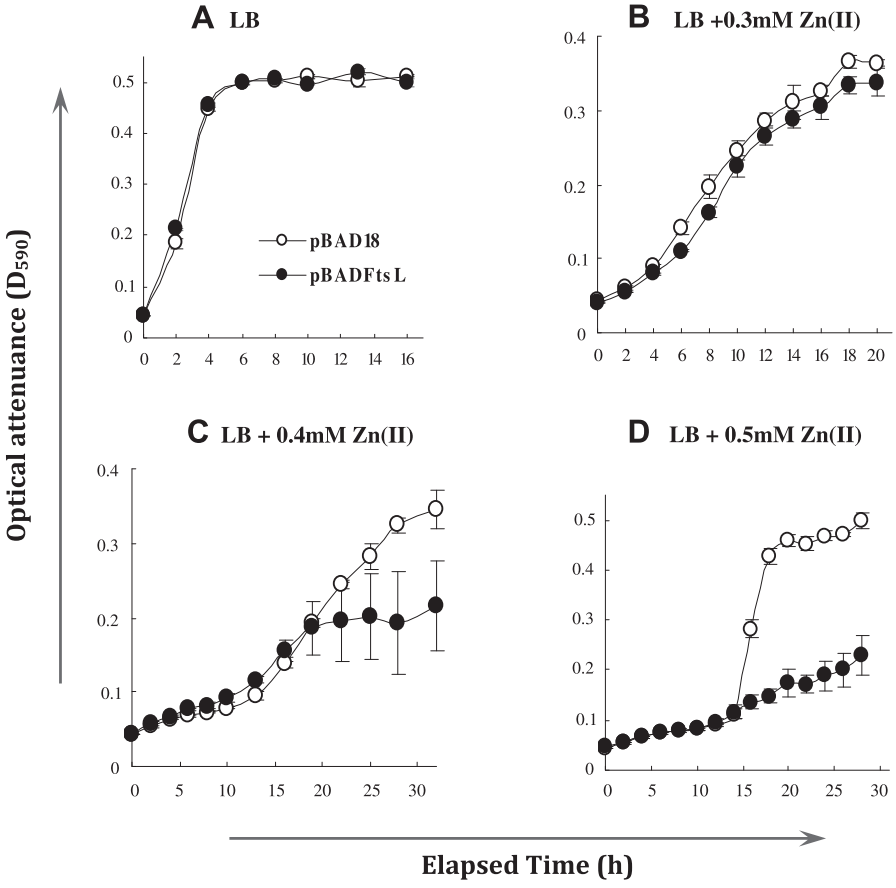

Fig. 3. The effect of *ftsL* expression on Zn(II) tolerance in *E. coli* cells grown in arabinose/Zn(II)-supplemented medium. *E. coli* (RW3110Δ*zntA*) cells containing either pBAD18 (open circles) or pBAD*ftsL* (closed circles) were cultured in LB media that contained arabinose (0.5% w/v) and a range of Zn(II)-ion concentrations. The growth of cultures was monitored periodically by optical attenuance (D_{590}) ($n = 3$).

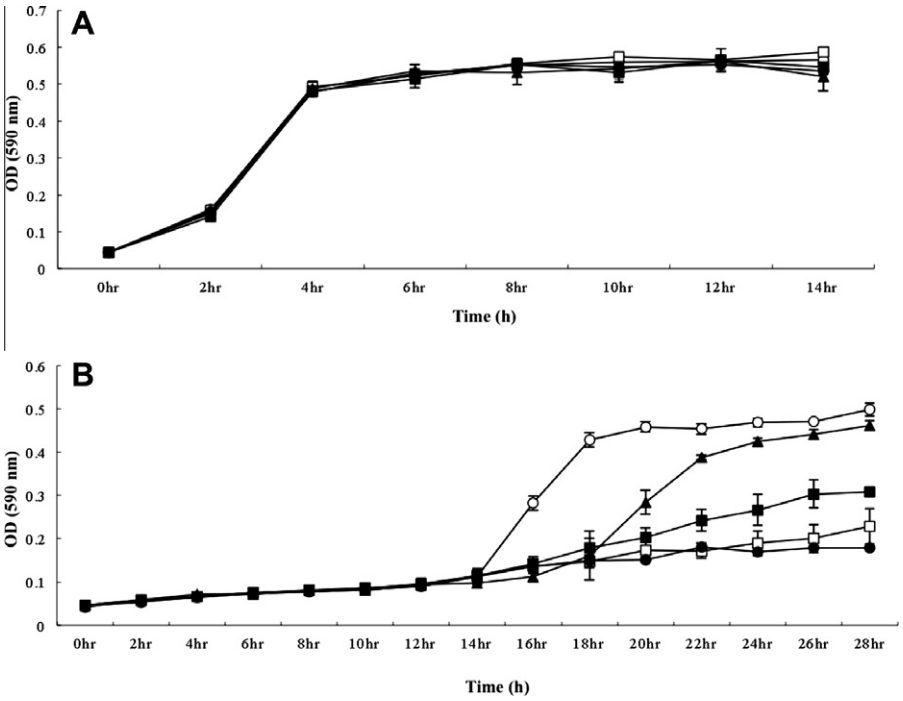

Fig. 4. The effect of *ftsL* expression on Zn(II) tolerance in *E. coli* cells grown in arabinose-supplemented medium (A) or arabinose/Zn(II)-supplemented medium (B). *E. coli* (RW3110Δ*zntA*) cells containing either pBAD18 (open circles), pBAD*ftsL* (open squares), pBAD*ftsL*$H_{19}H_{22}$ (closed circles), pBAD*ftsL*$C_{41}C_{45}$ (closed squares) and pBAD*ftsL*$H_{58}H_{59}$ (closed triangles) pBAD were cultured in LB media that contained arabinose (0.5% w/v) and a range of Zn(II) ion concentrations. The growth of cultures was monitored periodically by optical attenuance (D_{590}), ($n = 3$).

Table 2
The ability of FtsL mutant derivatives to complement *E. coli* (KS272Δ*ftsL*) for growth.

ftsL Derivative	LB medium	LB + 0.5 mM Zn(II)
w.t.	100 ± 5	100 ± 2
$H_{19}A,H_{22}A$	104 ± 1	76 ± 2
$C_{41}S,C_{45}S$	88 ± 2	18 ± 7
$H_{58}A,H_{59}A$	100 ± 1	74 ± 0

E. coli (KS272Δ*ftsL*) containing either pBAD*ftsL* (Cm), pBAD*ftsL* $H_{19}H_{22}$ (Cm), pBAD*ftsL*$C_{41}C_{45}$ or pBAD*ftsL* $H_{58}H_{59}$ (Cm) were grown to stationary phase in LB media with or without Zn(II) supplementation. The data presented are the mean average attenuance (D_{590}) reached by each strain and are shown as a percentage of the attenuance obtained by *E. coli* (KS272Δ*ftsL*):pBAD*ftsL* (Cm) cells, which was given an arbitrary value of 100%. Standard deviations are shown ($n = 3$).

The involvement of FtsL in the localisation of proteins to the septal machinery in the process of cell division represents the only function so far characterised for this protein. The results presented here demonstrate that this protein also has the capacity to confer Zn(II)-sensitivity upon *E. coli* (Table 1 and Fig. 3). Whilst expression of membrane proteins can be detrimental to cell growth and morphology, previous studies have demonstrated that the heterologous expression of *ftsL* from an inducible promoter does not confer any visible deviation from normal cell growth, cell physiology or cell length to *E. coli* cells [23] and indeed, our own data show that expression of *ftsL* from the P_{ara} promoter within the pBAD vector is not detrimental to cell growth. Therefore the alteration in Zn(II)-tolerance conferred by *ftsL* represents a novel function for this protein. Work carried out by the Beckwith laboratory has shown that GFP fusions to FtsL were targeted to the divisome during low expression; however, high-level expression e.g. in the presence of arabinose, caused FtsL to distribute randomly throughout the membrane [23]. We suggest that this random localisation could lead to the formation of FtsL multimers, or subsets of the cell division complex known to associate with FtsL, that may have the potential to mediate Zn(II)-import and confer the sensitive phenotype observed. Thus, the phenotype is indicative of the ability of FtsL to mediate Zn(II)-movement across the membrane.

Our model (Fig. 2B) would suggest that the sensitivity observed is due to FtsL-mediated Zn(II)-transfer across the membrane via an sulphur/imidazole associated 'bucket-brigade' mechanism. In order to test this hypothesis we determined the capacity of FtsL derivatives, lacking specific sulphur and imidazole groups, to direct the Zn(II)-sensitive phenotype. Replacement of the histidine pair ($H_{19,22}$) in the amino-terminal domain of FtsL resulted in the production of a derivative that conferred a growth pattern very similar to that of the negative control strain, having lost the ability to confer Zn(II)-sensitivity on the host. Replacement of the periplasmic histidine pair ($H_{58,59}$) had little effect on the growth characteristics and was functionally equivalent to w.t. *ftsL*. This suggests that these residues do not play a significant role in the generation of this phenotype. Replacement of the cysteine residues ($C_{41,45}$) with serine resulted in an intermediate phenotype showing some loss of Zn(II)-sensitivity. This data clearly demonstrates that the Zn(II)-sensitivity seen is an intrinsic property of FtsL and is consistent with a model in which the loss of the cytoplasmic HERH or the trans-membrane CLFIC motif affected the ability of FtsL to mediate Zn(II)-transfer across the cytoplasmic membrane. Thus, it is possible that these residues may play a role in the passage of Zn(II) across the cytoplasmic membrane.

Complementation of a chromosomally encoded *ftsL*-null mutation by each of the FtsL derivatives indicated that all of them could support the process of cell division (Table 2). So in optimal conditions, the residues targeted for alteration are not essential, although the loss of the cysteine pair ($C_{41,45}$) resulted in a diminished ability to complement (90% compared to w.t.) (Table 2). However, when cultured in Zn(II)-supplemented media, diminished complementation was seen for all of the derivatives but was particularly marked for that carrying the cysteine pair modification. Thus it is clear that the presence of residues alone is not essential for cell-division in optimal conditions, although, as mentioned above, this protein functions in concert with other sep-

tal-ring proteins and it is possible that some functional suppression of the mutations is occurring when in complex with the divisome. This data shows that these residues are important for FtsL function in non-optimal conditions, this diminished function is quite specific to Zn(II) with little or no response to Co(II), Ni(II) or Cu(II). We suggest that in enteric organisms, that experience transient increases in Zn(II)-exposure during passage through and out of the gut of animals, that FtsL has adapted either to become more resistant to these fluctuations or that Zn(II)-availability itself is being used as a signal in the regulation of the cell-division and that, in the absence of key residues, normal Zn(II)-trafficking is disrupted when Zn(II) is present at elevated concentrations. One intriguing publication has shown that in synchronously dividing *E. coli* cells, whilst the cellular concentration of metal ions such as Ca(II), K(I) and Mg(II) increase in a linear fashion with increasing bacterial cell numbers, the cellular content of Zn(II) increased in a stepwise manner throughout the cell cycle [24], with its cellular accumulation occurring at a key time point within the cell cycle, corresponding to the activation of FtsZ, and the initiation of both FtsA and murein synthesis. In this laboratory we have shown that growth of *E. coli* K12 in low-concentrations of Zn(II) confers a filamentous phenotype on cells, that is rapidly alleviated by the addition of Zn(II) to the growth medium [17]. Hence, the potential for cellular Zn(II)-status to act as one of a number of factors that co-ordinate the events of the *E. coli* cell cycle is evident.

All the results presented here are consistent with the hypothesis put forward by Beckwith and colleagues that FtsL plays a structural role in *E. coli* cell division [1,8,23]. It is suggested that FtsL, through the formation of densely packed multimers, may be involved in linking together the cytokinetic elements of *E. coli* cell division [8]. The data presented here are consistent with the designation of FtsL as a protein that can also mediate Zn(II)-sensitivity probably by increasing the permeability of the cytoplasmic membrane. This establishes a link between Zn(II)-modulation and a protein that is both integral and essential for cell division and although this does not seem to be essential for its role in cell division, this novel function for an integral and essential cell-division protein may have an overlying role in fine tuning the co-ordination of cell division in this prokaryotic system.

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References

[1] J. Errington, R.A. Daniel, D.J. Scheffers, Cytokinesis in bacteria, Microbiology and Molecular Biology Review 67 (2003) 52–56.
[2] N.W. Goehring, J. Beckwith, Diverse paths to midcell: assembly of the bacterial cell division machinery, Current Biology 15 (2005) 514–526.
[3] L.M. Guzman, J.J. Barondess, J. Beckwith, FtsL an essential cytoplasmic membrane-protein involved in cell-division in *Escherichia coli*, Journal of Bacteriology 174 (23) (1992) 7716–7728.
[4] L.M. Guzman, D.S. Weiss, J. Backwith, Domain-swapping analysis of FtsI, FtsL and FtsQ bitopic membrane proteins essential for cell division in *Escherichia coli*, Journal of Bacteriology 179 (16) (1997) 5094–5103.
[5] F. Ishino, H.K. Jung, M. Ikeda, M. Doi, M. Wachi, M. Matsuhashi, New Mutations Fts-36 Lts-33 and FtsW clustered in the Mra region of the *Escherichia coli* chromosome induce thermosensitive cell-growth and division, Journal of Bacteriology 171 (10) (1989) 5523–5530.
[6] M. Ueki, M. Wachi, H.K. Jung, F. Ishino, M. Matsuhashi, *Escherichia coli* MraR gene involved in cell-growth and division, Journal of Bacteriology 174 (23) (1992) 7841–7843.
[7] R.A. Daniel, E.J. Harry, V.L. Katis, R.G. Wake, J. Errington, Characterisation of the essential cell division gene *ftsL*(yllD) of *Bacillus subtilis* and its role in the assembly of the division apparatus, Molecular Microbiology 29 (2) (1998) 593–604.
[8] J.M. Ghigo, J. Beckwith, Cell division in *Escherichia coli*: role of FtsL domains in septal localization function and oligomerization, Journal of Bacteriology 182 (1) (2000) 116–129.
[9] M.D. Gonzalez, E.A. Akbay, D. Boyd, J. Beckwith, Multiple interaction domains in FtsL a protein component of the widely conserved bacterial FtsLBQ cell division complex, Journal of Bacteriology 192 (11) (2010) 2757–2768.
[10] C. Yanish-Perron, J. Viera, J. Messing, Improved M13 phage cloning vectors host strains - nucleotide sequences of the M13 MP18 and pUC18 vectors, Gene 33 (1985) 103–199.
[11] K.L. Strauch, J. Beckwith, An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins, Proceedings of the National Academy of Science USA 85 (5) (1988) 1576–1580.
[12] C. Rensing, B. Mitra, B.P. Rosen, The *zntA* gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase, Proceedings of the National Academy of Science USA 94 (1997) 14326–14331.
[13] L-M. Guzman, D. Belin, M.J. Carson, J. Beckwith, Tight regulation modulation and high-level expression by vectors containing the arabinose PBAD promoter, Journal of Bacteriology 177 (14) (1995) 4121–4130.
[14] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbour Laboratory Press, 1989.
[15] J.H. Miller, Experiments in Molecular Genetics, Cold Spring Harbor, NY, 1972.
[16] P. Barrineau, P. Gilbert, W.J. Jackson, C.S. Jones, A.O. Summers, S. Wisdom, The DNA sequence of the mercury resistance operon of the IncFII plasmid NR1, Journal of Molecular and Applied Genetics 2 (6) (1984) 601–619.
[17] D. Blencowe, A.P. Morby, unpublished.
[18] P.C. Bull, D.W. Cox, Wilson disease and Menkes disease: new handles on heavy-metal transport, Trends in Genetics 10 (1994) 246–252.
[19] S. Lutsenko, J.H. Kaplan, Organization of P-Type ATPases – significance of structural diversity, Biochemistry 34 (48) (1995) 15607–15613.
[20] C. Rensing, M. Ghosh, B.P. Rosen, Families of soft-metal-ion-transporting ATPases, Journal of Bacteriology 181 (19) (1999) 5891–5897.
[21] M. Solioz, C. Vulpe, CPx-type ATPases: a class of p-type ATPases that pump heavy metals, Trends in Biochemical Sciences 21 (7) (1996) 237–241.
[22] J.R. Wilson, C. Leang, A.P. Morby, J.L. Hobman, N.L. Brown, MerF is a mercury transport prote different structures but a common mechanism for mercuric ion transporters? FEBS Letters 472 (1) (2000) 78–82.
[23] J.M. Ghigo, D.S. Weiss, J.C. Chen, J.C. Yarrow, J. Beckwith, Localization of FtsL to the *Escherichia coli* septal ring, Molecular Microbiology 31 (2) (1999) 725–737.
[24] F.C. Kung, J. Raymond, D.A. Glaser, Metal ion content of *Escherichia coli* versus cell age, Journal of Bacteriology 126 (1976) 1089–1095.