Membrane topology of the N-terminus of the *Escherichia coli* FtsK division protein

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Abstract The *Escherichia coli* FtsK protein targets the septum, is essential for cell division and may play a role in DNA partitioning. Computer modelling suggests that the first 180 amino acids of the protein are embedded in the cytoplasmic membrane by up to six transmembrane domains. We demonstrate, using gene fusions, that the N-terminus contains four transmembrane helices that link two periplasmic domains. The first periplasmic domain contains an HEXXH amino acid sequence characteristic of zinc metalloproteases. We show by mutation analysis that the conserved glutamic acid of the HEXXH sequence is essential for FtsK function during septation.

Key words: Topology; Transmembrane; Cell division; FtsK; PhoA; Metalloprotease

1. Introduction

Formation of the *Escherichia coli* septum is co-ordinated by the action of a family of at least nine proteins that includes FtsZ, FtsA, FtsQ, FtsI, FtsL, FtsW, FtsN, FtsK and ZipA. Evidence suggests that these proteins may form a higher-order structure, the divisome or septalosome, which mediates the invagination of the cell envelope within a localised region at mid-cell. Several recent studies have examined the localisation and interactions of specific division proteins during biogenesis of the septum [1]. FtsZ plays a central role in cytokinesis; it aggregates into a ring at the future division site and remains at the leading edge of the invaginating membrane until cell division is completed. Genetic studies combined with immunofluorescence microscopy have indicated the likely order in which some of the division proteins localise to the Z-ring. Thus, ZipA appears at the division site at about the same time as FtsZ and FtsA, but its localisation is independent of FtsA and requires only FtsZ for localisation. [2]. Recruitment of FtsI is dependent on both FtsZ and FtsA while FtsN requires not only FtsZ and FtsA, but also FtsI and FtsQ [3–5].

A further protein, FtsK, is recruited to the division site at about the same time as FtsI and FtsN. Its localisation is dependent upon functional FtsZ and FtsA but requires neither FtsI nor FtsQ, suggesting that FtsK acts at an early step in septation [1]. The *ftsK* gene encodes a 147 kDa protein that has sequence similarity to a family of DNA translocating proteins that includes SpoIIIE of *Bacillus subtilis*. Genetic evidence suggests that FtsK may be bifunctional and play a dual role in cell division and chromosome localisation in *E. coli*. The N-terminal 202 codons of FtsK are essential and sufficient for cell division [1], while the C-terminal domain, which is highly homologous to SpoIIIE, may be required to ensure that replicated chromosomes are moved clear of the invaginating cell envelope. FtsK is predominantly associated with the membrane fraction, and has been localised to the division site by immunofluorescence microscopy and with GFP fusions [6].

An understanding of the topological organisation of FtsK and the other division proteins will be valuable in helping establish structure–function interrelationships not only between the division proteins, but also between the proteins and the membrane during assembly and contraction of the Z-ring. The hydropathy profile of the primary amino acid sequence of FtsK suggests a secondary structure in which the polytopic protein traverses the membrane through a series of α-helical transmembrane (TM) domains connected by hydrophilic loops. In the present study, the number and topology of candidate transmembrane segments within the cytoplasmic membrane has been investigated by creating a series of fusions between the N-terminus of FtsK and either β-galactosidase or alkaline phosphatase (reviewed in [7]).

Our study indicates that the N-terminus of FtsK contains four transmembrane domains that anchor the protein within the cytoplasmic membrane. The first periplasmic domain, delimited by TM1 and TM2, contains an HEXXH sequence characteristic of zinc metalloproteases (reviewed in [8]). Altering the glutamate residue of the sequence to alanine prevents FtsK from fulfilling its role in septation and suggests that FtsK may perform an enzymatic function at the division site.

2. Materials and methods

2.1. Growth media and strains

Bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* DH5α [9] was used to propagate and maintain the plasmids generated in this study. TOE44 [10] contains the temperature-sensitive *ftsK*44 cell division mutation and was used to test complementation. Chromosomal DNAs from *E. coli* TG1 and TOE44 were used for PCR amplification. Bacteria were routinely grown in Luria–Bertani (LB) medium with aeration at 37°C, except where otherwise indicated. Antibiotics were used at the following concentrations: ampicillin, 100 μg/ml and kanamycin, 30 μg/ml. Cells transformed with the β-galactosidase fusion vectors were selected on plates supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (30 μg/ml) and alkaline phosphatase fusion vectors on plates supplemented with 5-bromo-4-chloro-3-indolyl phosphate (30 μg/ml).

2.2. Molecular techniques and enzyme assays

Molecular techniques were performed according to standard protocols [11], or according to manufacturers' recommendations.

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Table 1
E. coli strains used in this study

Strain	Genotype	Source
DH5α	(endA-1 *hsdR*-17 ($r_k^-m_k^+$) *supE*-44 *thi*1 *recA*-1 *gyrA* (Nal^r) *relA*-1 Δ(*lacZYA-argF*) U169 [ϕ80dlacΔ(*lacZ*)M15]	[9]
TG1	F′ traD36 $lacI^q$ Δ ZM15 $proAB^+$/supE Δ(hsdM-mcrB)5 ($r_k^-m_k^+$ $mcrB^-$) thi Δ (lac-proAB)	Stratagene
TOE44	AB2497 *ftsK*44	[10]

2.3. Construction of pPhoA

The coding sequence of the *E. coli* alkaline phosphatase gene was amplified from TG1 using primers *pho*AP1*Hin*dIII and *pho*AP2 (Table 2). The resulting 1.3 kb fragment lacks the PhoA leader sequence (amino acids 1–22) but retains the PhoA termination codon. The fragment was digested with the restriction enzyme *Hin*dIII and cloned into the *lacZ* fusion plasmid pJBZ282 digested with *Hin*dIII and *Eco*RV, replacing the *lacZ* open reading frame with *phoA*, to obtain pPhoA. Selection for both pJBZ282 (encoding β-galactosidase) and pPhoA (encoding alkaline phosphatase) is kanamycin.

2.4. Construction of ftsK-lacZ and ftsK-phoA fusion vectors

Primer Lrp*Bam*HI P1 anneals beyond *lrp*, upstream of the *ftsK* regulatory region, and was used in conjunction with one of a series of downstream primers to generate PCR fragments with progressively greater lengths of *ftsK* DNA. (Table 3; the primers are numbered to indicate the last amino acid of FtsK encoded by the amplified fragment). Unique restriction sites for *Bam*HI and *Sal*I were incorporated at the 5′ and 3′ ends respectively, of the amplified DNAs. The PCR fragments were then cloned into pJBZ282 to create in-frame fusions between *ftsK* and *lacZ* and also into pPhoA to generate in-frame fusions with *phoA*.

2.5. Assay of β-galactosidase and alkaline phosphatase activities

DH5α cells transformed with either the *ftsK-lacZ* or *ftsK-phoA* fusions were grown to exponential phase, harvested and permeabilised with chloroform/SDS. β-Galactosidase and alkaline phosphatase activities were assayed by measuring the rates of hydrolysis of *o*-nitrophenyl-β-D-galactoside and *p*-nitrophenyl phosphate, respectively [12–14]. Activities are expressed as the average of at least triplicate cultures. The nucleotide sequences of the fusion junctions were confirmed by sequencing (Cambridge BioScience).

2.6. Modification of HEXXH sequence by mutagenic PCR

The Stratagene QuickChange site-directed mutagenesis protocol was used to introduce base substitutions into the *ftsK* gene of plasmids *ftsK*-P71, *ftsK*-P133, *ftsK*-L146 and pDDK20, resulting in the conversion of FtsK Glu-58 to Ala-58 in the HEXXH sequence of these plasmids. The mutation was introduced using the mutagenic PCR primers MutA-Glu-AlaP1 and MutA-Glu-AlaP2 (Table 2). After amplification with *pfu* polymerase (12 cycles at an annealing temperature of 55°C with a polymerisation time of 18 min), the PCR mixture was treated with *Dpn*I to degrade methylated parental template and also hybrid DNAs comprising mutant DNA annealed with methylated parental template. The reaction mix was used to transform ultracompetent cells (Stratagene). The resulting plasmids were sequenced to confirm that the mutation had been successfully introduced into the vectors (Cambridge BioScience).

2.7. Computer analysis

The putative membrane-spanning domains of the FtsK protein were identified from its sequence by hydrophobicity analysis using the TopPred2, SOSUI, TMPred, PHDhtm and the DAS transmembrane prediction servers [15–18]. Candidate membrane-spanning segments are predicted in Fig. 1A,B. Similarity searches were carried out using the Basic Local Alignment Search Tool (BLAST) from the Entrez server at NCBI and motif searches, using the Prosite Motif Search database [19].

3. Results

3.1. Predicted transmembrane orientation of FtsK

Several studies have shown that only about the first 200 amino acids of the N-terminus of the protein are needed for its proper action during cell division [1,6,20]. The theoretical number and orientation of membrane-spanning segments within the first 200 residues of FtsK was determined using computational algorithms to calculate the hydropathy profile, or helix orientation of the sequence. The five algorithms that were used in the analysis (TopPred2, TMPred, SOSUI, DAS and PHDhtm) predict that the protein contains between four and six possible membrane-spanning domains. Four transmembrane domains (domains I–IV, Fig. 1A,B) were predicted consistently by each of the five programs. A topology based on these data predicts that the four transmembrane domains would be linked by two periplasmic and a single cytoplasmic loop, with the N- and C-termini of the protein projecting into the cytoplasm. The SOSUI, DAS and PHDhtm programs

Table 2
Synthetic oligonucleotides used for amplification of *ftsK* sequence

Primer	Sequence	bp position on *ftsK* sequence
Lrp*Bam*HI P1	5′-CTCCCGTCAGGATCCCCTCTGC-3′	−1028
*pho*AP1*Hin*dIII	5′-CGAAGCTTCCGGACACCAGAAATGCCTG-3′	n/a
*pho*AP2	5′-CGGTTTTATTTCAGCCCCAGAGC-3′	n/a
Fusion 17	5′-CTCCAGAAGGCGGTCGACGCTAC-3′	+49
Fusion 47	5′-CGACCAGCTGTCGACCGAAGGC-3′	+134
Fusion 71	5′-CAGCGTGTCGACCAACCACGC-3′	+204
Fusion 94	5′-ACGCCAGTCGACCCAACAACC-3′	+276
Fusion 115	5′-CAAAACGTCGACGATGCGTAGCG-3′	+332
Fusion 133	5′-GATATCGTCGACGTTGATTGC-3′	+390
Fusion 139	5′-CCTGTCGACATACCAGATATC-3′	+405
Fusion 146	5′-GCTTAGTAAGTCGACAATGAC-3′	+432
Fusion 156	5′-CCCCCGCTACTGTCGACCAGTGG-3′	+465
Fusion 189	5′-CCAGCCGTCGACTTTTTCAGC-3′	+558
Fusion 218	5′-CTCTTCGTCGACATACTCATC-3′	+642
Fusion 250	5′-GAATTTGTCGACCAACCGTTT-3′	+741
MutA-Glu-AlaP1	5′-GGCCTGGCATGCACCTATCCATAATTTAGG-3′	+188
MutA-Glu-AlaP2	5′-CCTAAATTATGGATAGGTGCATGCCAGGCC-3′	+156

The position of the *Sal*I restriction site on each of the reverse primers is underlined.

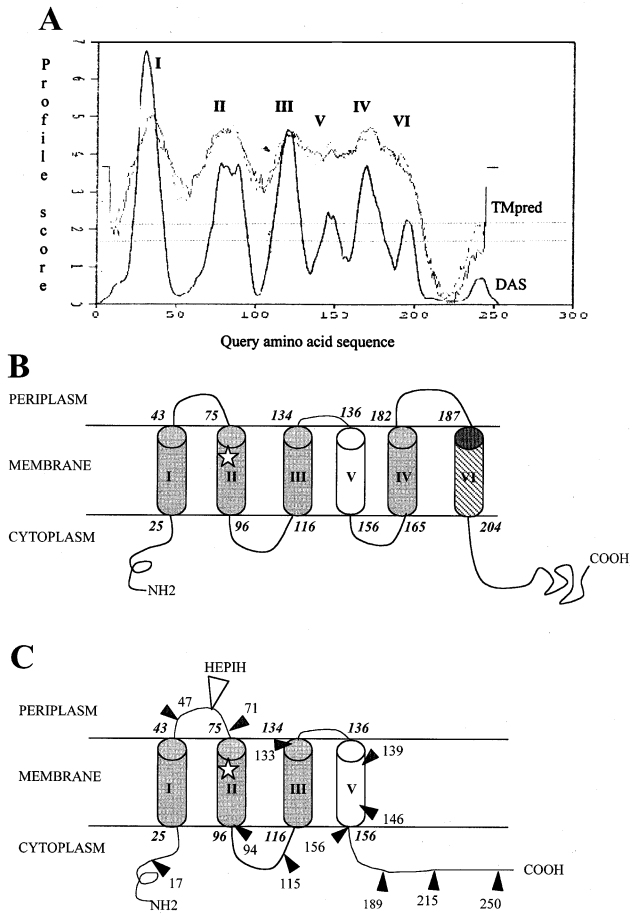

Fig. 1. A: Computer-generated output curves of membrane-spanning domains of FtsK predicted by TMPred (four domains) and DAS (six domains). B: Graphical representation of TM domains derived using TMPred, DAS, TopPred2, SOSUI and PHD. C: Topology of FtsK amino-terminus derived experimentally. The shaded cylinders (I–IV) represent the transmembrane domains predicted by all the modelling programs. Domain V was predicted by SOSUI, and domain VI by PHDhtm and DAS. The star indicates the location of the *ftsK44* mutation and the open arrow the location of the putative HEPIH metalloprotease signature. The shaded arrows correspond to the fusion points for constructs; their numbering corresponds to the last amino acid of FtsK in the fusion. The predicted first and last amino acids for each transmembrane domain are numbered and shown in italicised, bold typeface.

predict an additional transmembrane domain (V), localised to residues 136–156, which would orientate the C-terminus of the protein into the periplasm. The probability that this topology is taken up in vivo is low since recent studies have indicated that the C-terminal portion of the protein may be required for the resolution of chromosome dimers, a role that would be incompatible with it being located in the periplasm. However, in addition to the segment within residues 136–156, both the DAS and neural network PHDhtm programs suggest that a potential sixth transmembrane domain is located within residues 187–204 (VI, Fig. 1A,B). The existence of this domain in combination with domain V would, in principle, allow both the N- and C-termini to be located within the cytoplasm.

The computational approaches to identifying the membrane topology of FtsK suggest that at least four regions are likely to contain transmembrane helices, but do not clearly discriminate between the four and six TM domain models. To establish the number and orientation of transmembrane regions within FtsK, the β-galactosidase and alkaline phosphatase genes were fused to selected sequences within the 5′ end of *ftsK*.

3.2. Construction of LacZ and PhoA fusions to FtsK

PCR-derived 3′-truncated *ftsK* fragments were cloned into pJBZ282 to construct LacZ fusions, and into pPhoA for alkaline phosphatase fusions. The choice of fusion site was determined from hydrophobicity plots of FtsK, and selected so as to bond the carboxyl end of each putative TM domain to the reporter protein, either PhoA or LacZ. Ten fusions to each reporter protein were generated using this method (Table 3). In each construct, expression of the FtsK-LacZ/PhoA hybrid protein is under the control of the *ftsK* promoter. The enzyme activities of the hybrid proteins were determined in DH5α, and the cellular location of the C-terminal fusion predicted from the β-galactosidase or alkaline phosphatase activity of the resulting transformants. β-Galactosidase hybrids are active when fused to a cytoplasmic domain, whereas the corresponding PhoA fusions have little activity under these conditions; PhoA fusions are only fully active when the PhoA moiety is exposed to the periplasm. Constructs with an increasing number of amino acid residues might therefore be expected to exhibit a variable pattern of enzyme activity, reflecting the orientation of the protein as it traverses the membrane.

3.3. β-Galactosidase and alkaline phosphatase activities of hybrid proteins

The β-galactosidase and alkaline phosphatase activities of the resulting recombinant fusion constructs are presented in Table 3. The fusion protein FtsK-L17 is enzymatically active and likely to be located in the cytoplasm; the subsequent fusions, FtsK-L47 and FtsK-L71, are inactive, consistent with residues within the putative 25(22)–43 domain traversing the membrane, and positioning these fusions close to or within the periplasm. The alkaline phosphatase hybrid FtsKP-17 is inactive while both FtsKP-47 and FtsKP-71 are active, strongly supporting the predicted orientation. A 6–7-fold increase in β-galactosidase activity is observed on shifting the fusion junction from Leu-71 to Trp-94, and an almost 15-fold increase in activity when the length of FtsK fused to LacZ is extended to Arg-115. A further two fusions, FtsK-L133 and FtsK-L139, express low levels of β-galactosidase and high levels of alkaline phosphatase in accord with a periplasmic arrangement. Progressively longer fusions of up to 250 amino acids show high β-galactosidase activities (FtsK-L146, -L156, -L189, -L218, -L250) and low levels of alkaline phosphatase (FtsK-P146, -P156, -P189) and are predicted to be cytoplasmic. These data strongly favour a four, rather than a six, transmembrane topology for FtsK (Fig. 1C). However, the fourth transmembrane domain, responsible for reorienting the FtsK-L146 to FtsK-L250 C-terminal fusion proteins back into the cytoplasm, would not be the 165–182 segment (IV) identified in Model 1, but rather the 136–156 segment identified by the SOSUI algorithm (V). The cytoplasmic localisation of all fusion proteins from -L156 confirms the absence of further transmembrane segments within the amino-terminus of FtsK, the portion of the protein required specifically for cell division.

Table 3
Activities and deduced locations of the hybrid FtsK-PhoA and FtsK-LacZ proteins

Plasmid	pFtsK fusion site[a]	pFtsK-P series PhoA Activity (U[b])	pFtsK-L series β-Galactosidase activity (U[b])	Location[c]
pPhoA	–	–	–	–
pJBZ282	–	–	–	–
pFtsK-	17	14 ± 4	1598 ± 44	C
	47	170 ± 21	7 ± 4	P
	71	146 ± 9	65 ± 18	P
	94	11 ± 5	426 ± 21	C
	115	13 ± 8	942 ± 14	C
	133	177 ± 32	10 ± 3	P
	139	201 ± 39	12 ± 4	P
	146	17 ± 5	1057 ± 95	C
	156	16 ± 4	902 ± 18	C
	189	2 ± 2	922 ± 39	C
	218	–	611 ± 29	C
	250	–	801 ± 32	C
$pFtsK_{HAXXH}$ derivatives:				
	P71*	146 ± 5	–	P
	P133*	123 ± 5	–	P
	L146*	–	1393 ± 132	C
pDDK derivatives:				
	pDDK20	–	2260 ± 266	–
	$pDDK20_{HAXXH}$	–	2231 ± 40	–

P = periplasmic, C = cytoplasmic.
[a]The numbering of the fusions indicates the FtsK amino acid residue at which the sequence is fused to LacZ or PhoA. The fusion site was confirmed by DNA sequence analysis.
[b]Units of alkaline phosphatase or β-galactosidase expressed in Miller units.
[c]The topological orientation of FtsK indicated from the activities of the fusion proteins.

3.3. The ftsK44 mutation does not affect targeting at permissive temperatures

TOE44 (the thermosensitive *ftsK* mutant) divides normally in medium containing high salt (LB medium), but filaments in low salt medium at the restrictive temperature (42°C). Using FtsK-GFP fusion proteins, Yu et al. [6] reported that the mutant FtsK44 protein is unable to target the septum and suggested that this is responsible for the filamentation phenotype exhibited at 42°C. Surprisingly, they also reported that the FtsK44 protein is unable to localise the septum even at the permissive temperature. This result is inconsistent with the observation that TOE44 cells grow and divide normally at 30°C. We used two p*ftsK-phoA* derivatives (FtsK-P133K and FtsK-P139K), which contain the *ftsK44* mutation (Gly-80 to Ala-80) to test the targeting of the mutant FtsK44 protein to the membrane. Activities were measured from cells grown in both LB and L broth medium. The results in Table 4 indicate that the mutant protein retains its high alkaline phosphatase activities, even in low salt both at the permissive temperature and at 35°C (strains harbouring the mutant protein were not viable in L broth at temperatures above 35°C). The FtsK44-PhoA protein is therefore unaffected in its ability to target the membrane at permissive temperatures. To rule out the possibility that the FtsK44 protein associates with the membrane by interacting with wild type FtsK produced from the DH5α chromosomal locus, we tested both P133K and P139K in TOE44 in L broth at 35°C. The levels of alkaline phosphatase remained high (data not shown), indicating that even in the absence of wild-type FtsK protein, FtsK44 is able to target the membrane. This result conflicts with that reported by Yu et al. [6] but is in better keeping with the observed phenotype of TOE44 (i.e. no filamentation at permissive temperatures in low salt medium).

3.4. A zinc metalloprotease-like sequence lies in the N-terminal domain of FtsK

TOE44 can be specifically suppressed by inactivation of the *dacA* gene that encodes PBP5, a D-alanyl-D-alanine carboxypeptidase involved in cell wall synthesis in *E. coli*. It has previously been postulated that the suppression may result from the ability of FtsK to utilise the unmodified pentapeptide side chains exposed on the peptidoglycan within the periplasm [10]. We therefore considered the possibility that structural features within the membrane-associated domains of FtsK may be required for full FtsK activity. A Prosite Motif Search indicated that the periplasmic domain connecting transmembrane segments 1 and 2 contains a sequence of amino acids, HEPIH (His-57 to His-61), that resembles the HEXXH signature motif of zinc metalloproteases. Within the conserved metalloprotease motif, the two histidine residues bind a zinc atom while the glutamic acid is the catalytic centre of the molecule in protein hydrolysis. This highly conserved glutamate residue (E) is essential for activity; site-directed mutation of the residue abolishes the proteolytic activity of the purified proteins [21–23].

To investigate the possible importance of this motif in FtsK, we carried out PCR-mediated site-directed mutagenesis on the conserved glutamic acid residue (Glu-58) in the HEPIH sequence. We then examined the impact of its loss on *ftsK* transcription, on the topology of the protein and on the ability of FtsK to participate in septum formation (by analysing its ability to complement the *ftsK44*(Ts) mutation).

3.5. The HEXXH motif is essential for FtsK to function in cell division

The possible function of the Glu-58 residue in FtsK activity was investigated by substituting it with an alanine residue

Table 4
Alkaline phosphatase activities of modified *ftsK* fusions with varying salt conditions

	LB (high salt)	L broth (low salt)		
	37°C	30°C	35°C	42°C
P71	146 ± 9	164 ± 9	–	275 ± 29
P71*	146 ± 5	153 ± 2	–	185 ± 23
P133	177 ± 32	118 ± 18	–	148 ± 21
P133*	123 ± 5	104 ± 6	–	173 ± 48
P133K	304 ± 12	248 ± 35	357 ± 50	–
P139K	230 ± 40	191 ± 54	236 ± 35	–

An asterisk indicates modification of the FtsK sequence from HEXXH to HAXXH in the corresponding plasmid. Constructs P133K and P139K contain the *ftsK44* mutation within their cloned sequence. A dash shows the construct was not tested under the conditions indicated.

(Ala-58) by mutagenic PCR in the fusion vectors FtsK-P71, FtsK-P133 and FtsK-L146. We first examined the ability of the mutant proteins to target the membrane and assume their normal topologies within the cytoplasmic membrane. The enzyme activities of the mutant fusion proteins (indicated by an asterisk) were assayed and found to be equivalent to the wild-type fusions (Table 3), confirming that under standard growth conditions the amino acid substitution does not appear to alter the topologies of the resulting proteins. For example, the activity of the FtsK-P71* fusion remained at 146 units after mutagenesis, consistent with the hybrid protein retaining its ability to span the membrane and allow translocation of PhoA into the periplasm. Since the phenotype of the *ftsK44*(Ts) mutant is only apparent at elevated temperature and low salt concentrations, we also examined the activities of the FtsK-P71* and FtsK-P133* constructs in L-broth (no salt) at 30°C and 42°C (Table 4). Cells transformed with either FtsK-P71* or FtsK-P133* do not exhibit a reduction in their enzyme activities, supporting the conclusion that the mutant protein has lost neither its ability to target the membrane nor to assume its correct topology within the membrane.

To test the effect of mutation of the putative metal binding motif on the ability of the modified protein to complement TOE44, we introduced the same Glu-58 to Ala-58 conversion into the *lacZ* vector pDDK20 (R. Dorazi, unpublished). This vector contains the first 1011 nucleotides of the *ftsK* coding sequence along with 750 bp of its adjacent regulatory region in transcriptional fusion with *lacZ*, and is able to complement the *ftsK44*(Ts) mutation at 42°C in absence of salt. PDDK20 expresses β-galactosidase to high level (2600 units). Table 4 shows that while the enzymatic activity for $pDK20_{HAXXH}$ is comparable to that observed for the original construct (2300 ± 40 units compared to 2600 ± 266 units), this construct is unable to complement TOE44.

Altogether, these results indicate that the mutation has no direct effect either on the topology or on the transcription activity of *ftsK*, but that it *does* affect the ability of the protein to function in septation. These results suggest that the glutamic acid residue within the putative metalloprotease motif is of significant functional importance for FtsK activity in cell division.

4. Discussion

This study examined the topology of the FtsK cell division protein by creating C-terminal fusions between *ftsK* and *lacZ* and between *ftsK* and *phoA*. The data generated from the two series gave closely matched, reciprocal activities and suggest that FtsK is anchored within the cytoplasmic membrane by four N-terminal transmembrane domains. Two periplasmic loops and a single cytoplasmic loop interconnect the membrane-spanning segments. The arrangement of transmembrane domains I–III is in agreement with that predicted by computer modelling. The fourth domain corresponds to the unique domain identified by the SOSUI algorithm (V), which would suggest that domains III and V are connected by only a very short periplasmic loop. Fusions -L189 to -L250 are all cytoplasmic, indicating that the putative domains IV and VI do not exist. Additional transmembrane domains may be located further into the C-terminal part of the protein, but neither hydropathy nor structural analyses of the protein support this idea. Further domains would have to be arranged so as to position the carboxy-terminus of the protein into the cytoplasm, since a periplasmic location would be inconsistent with its reported role in the resolution of chromosome dimers.

Our results also suggest that sequences within the N-terminus of FtsK confer functionality on the protein. The presence of an HEXXH amino acid sequence, similar to the signature motif of the zinc metalloprotease enzymes, within the first periplasmic domain of the protein is required for FtsK to function in septum formation. Mutation of the glutamic acid residue, the catalytic centre in zinc metalloproteases, is sufficient to prevent TOE44 complementation without changing either the topology of the new protein or its level of transcription, perhaps by modifying an enzymatic activity associated with the protein. Although speculative, the location of the motif within the periplasmic domain may support a role for FtsK in septal peptidoglycan biosynthesis during cell division, as suggested by the earlier observation that the TOE44 phenotype is suppressed by deletion of *dacA*, encoding the peptidoglycan-modifying enzyme PBP5 [10].

The hybrid proteins expressed from the fusion vectors, with the exception of -L218 and -L250, are unlikely to be able to express functional FtsK since a minimum of about 205 amino acids is required for the protein to be active in septation. Interestingly, none of the constructs are lethal when the cells are grown at 37°C in the presence of salt. This indicates that the chromosomally encoded FtsK targets the septum and performs its role in cell division despite the large excess of non-functional protein that is also targeting the membrane. However, in the absence of salt and at elevated temperatures (42°C), cells carrying these fusions show sign of filamentation, which may result from competition between the natural FtsK and the truncated hybrids.

The mutation responsible for the thermosensitive FtsK44 phenotype is a simple transversion that changes a glycine into an alanine residue at position 80, inside the second transmembrane domain. Computer modelling suggests that far from disrupting the topology of the segment, this conservative mutation would in fact increase its stability. Nonetheless, a previous study investigating the localisation of FtsK using FtsK-GFP fusions has shown that the FtsK44 protein is unable to target the septum at either permissive or restrictive temperatures [6]. It was concluded that the protein remains associated with the cytosol, which is surprising since there is no obvious defect in cell division when the strain is cultured in low salt medium at 30°C. We demonstrate that the FtsK44 mutant protein *is* able to target the membrane at permissive

temperatures, although we cannot distinguish how that localisation is achieved. Thus, while the mutant protein may simply retain its wild-type transmembrane configuration it is also possible that the FtsK44 mutation disrupts localisation of domains II and I, so that targeting is effected solely through domain III. The difference between our observations and those of Yu et al. [6] may simply result from an intrinsic instability in the longer GFP fusions, with the resulting accumulation of the breakdown products in the cytoplasm. Crucially, the phenotype of TOE44 at the permissive temperature is clearly consistent with FtsK44 being able to target the membrane in low salt at the permissive temperature.

This study provides evidence for the in vivo topology of the FtsK protein and demonstrates that previously uncharacterised features within the protein may contribute to its function during the biogenesis of the septum.

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