Three functional subdomains of the *Escherichia coli* FtsQ protein are involved in its interaction with the other division proteins

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FtsQ, an essential protein for the *Escherichia coli* divisome assembly, is able to interact with various division proteins, namely FtsI, FtsL, FtsN, FtsB and FtsW. In this paper, the FtsQ domains involved in these interactions were identified by two-hybrid assays and co-immunoprecipitations. Progressive deletions of the *ftsQ* gene suggested that the FtsQ self-interaction and its interactions with the other proteins are localized in three periplasmic subdomains: (i) residues 50–135 constitute one of the sites involved in FtsQ, FtsI and FtsN interaction, and this site is also responsible for FtsW interaction; (ii) the FtsB interaction is localized between residues 136 and 202; and (iii) the FtsL interaction is localized at the very C-terminal extremity. In this third region, the interaction site for FtsK and also the second site for FtsQ, FtsI, FtsN interactions are located. As far as FtsW is concerned, this protein interacts with the fragment of the FtsQ periplasmic domain that spans residues 67–75. In addition, two protein subdomains, one constituted by residues 1–135 and the other from 136 to the end, are both able to complement an *ftsQ* null mutant. Finally, the unexpected finding that an *E. coli ftsQ* null mutant can be complemented, at least transiently, by the *Streptococcus pneumoniae divIB/ftsQ* gene product suggests a new strategy for investigating the biological significance of protein–protein interactions.

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INTRODUCTION

The development of a division machinery (divisome) constituted by various proteins, which form macromolecular complexes, is necessary for bacterial cell division. Comparison of different bacterial genomes has shown that the division genes are well conserved among bacteria, indicating that most bacterial groups share common division machinery and mechanisms. Nevertheless, none of the known division genes is present in all the phylogenetic groups (for reviews, see Margolin, 2000; Weiss, 2004; Vicente & Rico, 2006; Vicente *et al.*, 2006).

In *Escherichia coli*, cell division requires the assembly of at least 15 proteins at the mid-cell (Buddelmeijer & Beckwith, 2002; Bernhardt & de Boer, 2003). These proteins belong to various functional groups that participate in invagination, constriction of the three envelope layers and separation of the two daughter cells. Among these proteins, 10 are essential under standard laboratory conditions and their localization occurs according to a largely linear hierarchy: FtsZ > (FtsA, ZipA) > FtsK > FtsQ > (FtsL, FtsB) > FtsW > FtsI > FtsN.

Within this hierarchy, a given protein requires all upstream proteins (to the left) to localize and is, in turn, required for the localization of proteins further downstream (to the right) (Chen & Beckwith, 2001; Buddelmeijer & Beckwith, 2002).

To initiate cell division, the GTP-binding tubulin-like FtsZ protein forms an intracellular ring at the division site localized equidistant between the two cell poles, by FtsZ monomer self-assembly (Lutkenhaus & Addinall, 1997; Lowe & Amos, 1998; for a review, see Errington *et al.*, 2003). The Z-ring, besides its dynamic role in cytokinesis (assembly at the beginning of the division process and disassembly at the end of the division process) serves as a cytoskeletal scaffold for the recruitment of proteins of the cell division machinery (Bi & Lutkenhaus, 1991; Romberg & Levin, 2003).

Once both FtsA and ZipA are in place, the remaining proteins are recruited. These include bitopic (FtsQ, FtsL, FtsB, FtsN, FtsI) and polytopic (FtsK, FtsW) membrane proteins. The first group is characterized by a short cytoplasmic N-terminus, a single transmembrane segment and a large periplasmic domain (Guzman *et al.*, 1992, 1997; Dai *et al.*, 1996; Bowler & Spratt, 1989). In the second group, FtsK is a large protein, highly conserved throughout the

Abbreviations: MSS, membrane-spanning segment; POTRA, polypeptide transport-associated; THA, two-hybrid assay; TP, two phages.

eubacteria, with distinct roles in cell division and in chromosome segregation (Wang & Lutkenhaus, 1998) and FtsW, a member of the SEDS (shape, elongation, division and sporulation) family of proteins (Ikeda *et al.*, 1989; Henriques *et al.*, 1998). AmiC and EnvC are two peptidoglycan hydrolases that localize to the septal ring and play an important role in the separation of daughter cells (Bernhardt & de Boer, 2003).

The mechanism(s) governing the localization of these late proteins, and how they give rise to the assembly pathway, remain poorly understood. In fact several findings indicate that the pathway may not be as linear as first expected. Bacterial two-hybrid analysis suggests a highly interconnected network of proteins (Di Lallo *et al.*, 2003; Karimova *et al.*, 2005), and at least some cell division proteins assemble independently of their normal association with the divisome (Buddelmeijer & Beckwith, 2004; Goehring *et al.*, 2005). Moreover, several of the division components, including ZipA and FtsK, can be completely bypassed via suppressor mutations or overexpression of other divisome components (Geissler *et al.*, 2003; Geissler & Margolin, 2005).

Goehring *et al.* (2005) have recently developed a method to circumvent the need for pre-assembly of upstream proteins at the ring for the recruitment of downstream ring components. The first results obtained using this procedure indicated that FtsQ can coordinate the recruitment of the downstream proteins, except FtsN, in the absence of the upstream FtsA and FtsK proteins (Goehring *et al.*, 2005). It was also observed that prematurely localized FtsQ can back-recruit FtsK even in the absence of the upstream protein FtsA. This observation suggested a direct protein-protein interaction between FtsQ and FtsK in the division complex and was the first *in vivo* assay to show back-recruitment of division proteins.

Understanding how all these proteins interact with each other is crucial to elucidate the molecular mechanism of their recruitment and assembly at the division site. The interaction web among FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsL, FtsW, FtsI and FtsN was depicted by means of the two-hybrid assay (Di Lallo *et al.*, 2003). The interaction between FtsB and FtsQ is described in this paper.

This interaction web is complex because some division proteins, such as FtsQ, the subject of this work, have many partners. FtsQ, besides homodimerizing, interacts with FtsI, FtsL, FtsN, FtsK, FtsW (Di Lallo *et al.*, 2003; Karimova *et al.*, 2005) and FtsB (this work). Interactions between FtsQ, FtsL and FtsB were recently confirmed by biochemical methods (Buddelmeijer & Beckwith, 2004), and interactions between FtsQ and FtsI, and between FtsL and FtsN, by another prokaryotic two-hybrid assay (Karimova *et al.*, 2005).

FtsQ (Chen *et al.*, 1999) is a protein with a short (24 aa) cytoplasmic N-terminus, a single (25 aa) transmembrane segment, and a large (227 aa) periplasmic domain (Carson *et al.*, 1991; Guzman *et al.*, 1997). It has a polypeptide

transport-associated (POTRA) domain which is functionally associated with protein interactions or chaperone function (Sanchez-Pulido *et al.*, 2003). In *E. coli*, FtsQ assembles with FtsL and FtsB into a trimeric complex before their localization (Buddelmeijer & Beckwith, 2004), while in *Streptococcus pneumoniae* their homologues form a transient complex during septation (Noirclerc-Savoye *et al.*, 2005).

Despite its high number of interactions with the other division proteins and its role in localization of some of them, the exact role of FtsQ is still unknown. It has been proposed that FtsQ provides a structural link between early- and late-assembling divisomal components (Di Lallo *et al.*, 2003) and could regulate the assembly of these division proteins at the division site and the activity of the peptidoglycan assembly machinery within the divisome (Piette *et al.*, 2004). In addition, it was recently proposed that the FtsQ POTRA domain might function as a chaperone that specifically recognizes secretion- or assembly-competent forms of these polypeptides, suggesting that FtsQ could have a role in controlling the correct assembly of the divisome.

The aim of this work was not only to identify the FtsQ domains involved in the interaction with the other partner proteins but also to investigate the biological significance of these interactions in order to elucidate their assembly and participation in the divisome construction by also exploiting the possible capacity of orthologous proteins to complement an *E. coli* *ftsQ* null mutant.

METHODS

Media and chemicals. LB broth for bacterial culture and plating and SM (salt solution) for bacteria dilutions were as described by Miller (1972). The antibiotics used (Sigma) were ampicillin (50 µg ml⁻¹), chloramphenicol (34 µg ml⁻¹) and kanamycin (30 µg ml⁻¹). Synthetic oligonucleotides used in this work are listed in Table 1.

Bacterial strains and plasmids. Bacterial strains (all *E. coli* K-12 derivatives) and plasmids used in this work are listed in Table 2. Recombinant plasmids were constructed by cloning the genes (or gene fragments) of interest in the *SalI* and *BamHI* restriction sites of pCIP₂₂ and pCIP₄₃₄ vectors. The DNA of the gene of interest was obtained by PCR amplification using specific oligonucleotides carrying compatible restriction sites at the ends. All the constructs were controlled by DNA sequencing.

β-Galactosidase assay and two-hybrid assay. β-Galactosidase activity was assayed as described by Miller (1972). The two-hybrid assay was performed on bacterial cultures grown to OD₆₀₀ 0.5 at 34 °C in LB medium supplemented with 1 × 10⁻⁴ M IPTG, as described previously (Di Lallo *et al.*, 2001).

General microbiological and recombinant DNA techniques. Standard microbiological techniques were as described by Miller (1972). Standard procedures were used for small-scale plasmid preparations, endonuclease digestion, ligation, agarose gel electrophoresis, the elution of DNA fragments from agarose and bacterial transformation (Sambrook *et al.*, 1989). PCR was carried out using the *Taq* DNA polymerase kit (Promega), according to the recommendations of the manufacturer.

Table 1. Synthetic oligonucleotides used in this work

No.	Name	Sequence	Gene	Pos.	Site(s)
1	FtsQ 1F	5'-CGGTCGACCATGTCGCAGGCTGC-3'	<i>ftsQ</i>	-1	<i>Sall</i>
2	FtsQ 1F	5'-AACTGCAGATGTCGCAGGCTGCTCTG-3'	<i>ftsQ</i>	-1	<i>PstI</i>
3	FtsQ 61F	5'-GCGTCGACTAATGAACGCGTTGGCG-3'	<i>ftsQ</i>	61	<i>Sall</i>
4	FtsQ 96F	5'-GCGTCGACCGTTTAAACGACAGTG-3'	<i>ftsQ</i>	96	<i>Sall</i>
5	FtsQ 136F	5'-GCGTCGACCTTGGGCTGGATGGAAGATG-3'	<i>ftsQ</i>	136	<i>Sall</i>
6	FtsQ 148F	5'-GCTCTAGGGATGCGCAACGCCTGC-3'	<i>ftsQ</i>	148	<i>XbaI</i>
7	FtsQ 409F	5'-GCTCTAGACATGGTAGACGCGGAAG-3'	<i>ftsQ</i>	409	<i>XbaI</i>
8	FtsQ 409F	5'-TTGCGGCCGCTCGAGGTGGCACAACGAGAT-3'	<i>ftsQ</i>	409	<i>NotI</i> , <i>XhoI</i>
9	FtsQ 72R	5'-AACTGCAGACGCGTTCCATTATTGCGGC-3'	<i>ftsQ</i>	72	<i>PstI</i>
10	FtsQ 147R	5'-GAAGATCTTCACATCCAGCCCAACACGAC-3'	<i>ftsQ</i>	147	<i>BglII</i>
11	FtsQ 147R	5'-GCTCTAGACATCCAGCCCAACACGACCC-3'	<i>ftsQ</i>	147	<i>XbaI</i>
12	FtsQ 171R	5'-GAAGATCTTCAGAGCGGCAGGCGTT-3'	<i>ftsQ</i>	171	<i>BglII</i>
13	FtsQ 172F	5'-GCTCTAGATCAAAGCTGGTGTGACCGG-3'	<i>ftsQ</i>	172	<i>XbaI</i>
14	FtsQ 213R	5'-GAAGATCTTCAATTACGTGTGTAATGGCG-3'	<i>ftsQ</i>	213	<i>BglII</i>
15	FtsQ 315R	5'-GAAGTCTTCCACGGCAGGCGTTGTC-3'	<i>ftsQ</i>	315	<i>BglII</i>
16	FtsQ 408R	5'-GAAGATCTTCAATGTTGATCATTCCACCGC-3'	<i>ftsQ</i>	408	<i>BglII</i>
17	FtsQ 408R	5'-TTGCGGCCGCTCGAGTCAGTGGACAACGAGTTATG-3'	<i>ftsQ</i>	408	<i>NotI</i> , <i>XhoI</i>
18	FtsQ 606R	5'-GAAGATTTACGTCAACTGCCAGGAAC-3'	<i>ftsQ</i>	606	<i>BglII</i>
19	FtsQ 702R	5'-GAAGATCTTCAGTTGCCATCGGTTGC-3'	<i>ftsQ</i>	702	<i>BglII</i>
20	FtsQ 831R	5'-GAAGATCTTCATTGTTGTTCTGCCTGTG-3'	<i>ftsQ</i>	831	<i>BglII</i>
21	FtsQ 831R	5'-CGGGATCCTCATTGTTGTTCTGCCTGTG-3'	<i>ftsQ</i>	831	<i>BamHI</i>
22	FtsQ 831R	5'-CCAAGCTTTTCATTGTTGTTCTGCCTGTG-3'	<i>ftsQ</i>	831	<i>EcoRI</i>
23	FtsB 1F	5'-GCGTCGACCATGGTAACTAACGCTG-3'	<i>ftsB</i>	-1	<i>Sall</i>
24	FtsB 312R	5'-CGGGATCCTTATCGATTGTTTTGCC-3'	<i>ftsB</i>	312	<i>BamHI</i>
25	FtsN 1F	5'-TTGCGGCCGCTCGAGGTGGCACAACGAGATTATG-3'	<i>ftsN</i>	-1	<i>NotI</i> , <i>XhoI</i>
26	FtsN 159R	5'-GCTCTAGAGAAGTACAGACCACCGTAAGG-3'	<i>ftsN</i>	159	<i>XbaI</i>
27	FtsN -250F	5'-AACTGCAGGATCCGCGTGACAACCATC-3'	<i>ftsN</i>	-250	<i>PstI</i>
28	FtsW 226R	5'-CGGGATCCTCAGTTGGTTAAGCGTTGCCCTA-3'	<i>ftsW</i>	226	<i>BamHI</i>
29	FtsW 933R	5'-GGATCCTCAACCGACATACCCAGTTCTTCGC-3'	<i>ftsW</i>	933	<i>BamHI</i>
30	FtsW 534R	5'-GGATCCTCAGAAGCCGCGCAGGTTATTACGT-3'	<i>ftsW</i>	534	<i>BamHI</i>
31	FtsW 226R	5'-CGGGATCCTCAGTTGGTTAAGCGTTGCCCTA-3'	<i>ftsW</i>	226	<i>BamHI</i>
32	MalF 48F	5'-GCGTCGACATGGTCAGTGCTAGGTCT-3'	<i>malF</i>	48	<i>Sall</i>
33	MalF 49F	5'-AACTGCAGTGGTCGTGCTAGGCTT-3'	<i>malF</i>	49	<i>PstI</i>
34	MalF 108R	5'-GCTCTAGGCGTACATTAACAAC-3'	<i>malF</i>	108	<i>XbaI</i>

Construction of *ftsQD8*. In order to obtain a mutated FtsQ protein in which 8 aa, in the region between 147 and 171 bp of the gene sequence, were deleted and replaced by 2 aa derived by religation of the *XbaI* site, two *ftsQ* fragments (1–147 bp and 148–831 bp) were amplified with oligos 1 and 10, and 6 and 19, respectively. After purification and digestion with *XbaI*, the two DNA fragments were joined together with T4 DNA ligase and then reamplified with oligos 1–19. The PCR fragment obtained was purified, digested with *Sall* and *BglII* and inserted into plasmid *pCp22* digested with *Sall* and *BamHI*. The construct was then confirmed by DNA sequencing.

Construction of Q1Q2 plasmid. For the construction of plasmid *pCp22-ftsQ1Q2*, the *ftsQ* gene was divided into two fragments, Q1 and Q2 (Fig. 1). The Q1 fragment (1–408) was amplified by PCR using primers 1 and 16; the resulting fragment contains the *Sall* site at the 5' end and the *PstI* and *NotI* sites at the 3' end. The Q2 fragment (409–831 bp), amplified with primers 8 and 20, contains the *NotI* and *XhoI* sites at the 5' end and the *BglII* site at the 3' end. After digestion with *NotI*, the two fragments were joined together with T4 DNA ligase and reamplified with the external primers 1 and 20.

After digestion with *Sall* and *BglII*, the construct Q1Q2 was cloned in *pCp22* vector digested with *Sall/BamHI*, yielding the plasmid *pQ1-408Q2409-831*. For the correct expression and membrane localization of Q2 fragment, the DNA sequence encoding the cytoplasmic and transmembrane domains of FtsN (1–159 bp) was amplified with oligos 26 and 27 and cloned in *pQ1-408Q2409-831* between the Q1 and Q2 sequences. The FtsN fragment also contains the Shine–Dalgarno (SD) sequence for the appropriate translation of the FtsN₁₋₁₅₉-Q2 construct. After digestion with *PstI* and *XhoI*, the fragment was cloned in *pQ1-408Q2409-831* digested with the same enzymes, yielding plasmid *pCp22-ftsQ1Q2*.

For the construction of plasmid *pCp22-ftsQ2*, containing only the Q2 fragment, the sequence FtsN₁₋₁₅₉-Q2 was amplified from *pCp22-ftsQ1Q2* with primers 25 and 20, digested with *PstI* and *BglII* and cloned into the vector *pCp22* previously digested with the same enzymes. The constructs were confirmed by DNA sequencing.

Construction of GFP and GST derivatives for co-immunoprecipitation studies. The division proteins fused with GFP (green fluorescent protein of *Aequorea victoria*) were obtained by in-frame cloning of the PCR-produced DNA fragment of the corresponding

Table 2. Bacterial strains and plasmids

Strains	Relevant genotype	Source
71/18	<i>supE thy Δ(lac-proAB) F' [proAB⁺ lacI^q lacZΔM15]</i>	Dente <i>et al.</i> (1983)
R721	71/18 <i>glpT::O-P_{434/P22} lacZ</i>	Di Lallo <i>et al.</i> (2001)
JOE170	KS272/ <i>ftsQ::Tnpho450</i> (Kan ^r)/pJC10	Chen <i>et al.</i> (1999)
αDH5	<i>supE44 ΔlacU169 hsdR17 recA1 endA1 gyrA96 relA1</i>	Lab. collection
Plasmids		
pJC10	pBAD33- <i>ftsQ</i>	Chen <i>et al.</i> (1999)
pBAD33	Arabinose regulation, Cm ^r , pACYC184 origin	Guzman <i>et al.</i> (1995)
pCI _{P22}	pC132 derivative carrying NH ₂ end of P22 repressor	Di Lallo <i>et al.</i> (2001)
pCI ₄₃₄	pACYC177 derivative carrying NH ₂ end of 434 repressor	Di Lallo <i>et al.</i> (2001)
pCI _{P22} - <i>ftsQ</i>	pCI _{P22} derivative carrying the <i>ftsQ</i> gene	Di Lallo <i>et al.</i> (2003)
pCI _{P22} - <i>ftsQ</i> _{61–831}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 61 to 831 bp	This work
pCI ₂₂ - <i>ftsQ</i> _{61–702}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 61 to 702 bp	This work
pCI _{P22} - <i>ftsQ</i> _{61–606}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 61 to 606 bp	This work
pCI ₂₂ - <i>ftsQ</i> _{1–407}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 1 to 407 bp	This work
pCI _{P22} - <i>ftsQ</i> _{1–315}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 1 to 315 bp	This work
pCI ₂₂ - <i>ftsQ</i> _{1–215}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 1 to 215 bp	This work
pCI _{P22} - <i>ftsQ</i> _{1–171}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 1 to 171 bp	This work
pCI ₂₂ - <i>ftsQ</i> _{1–147}	pCI _{P22} derivative carrying the <i>ftsQ</i> gene fragment from 1 to 147 bp	This work
pCI _{P22} -QFQ	pCI _{P22} derivative carrying the Q _{Cyt} MalF _{MSS} Q _{peripl} swap	This work
pCI _{P22} -FQ	pCI _{P22} derivative carrying the MalF _{MSS} Q _{peripl} swap	This work
pCI _{P22} - <i>ftsQ</i> 1Q1Q2	pCI _{P22} derivative carrying the Q1Q2 construct	This work
pCI _{P22} - <i>ftsQ</i> 1	pCI _{P22} derivative carrying the Q1 construct	This work
pCI _{P22} - <i>ftsQ</i> 2	pCI _{P22} derivative carrying the Q2 construct	This work
pCI ₄₃₄ - <i>ftsN</i>	pCI ₄₃₄ derivative carrying the <i>ftsN</i> gene	This work
pCI ₄₃₄ - <i>ftsQ</i>	pCI ₄₃₄ derivative carrying the <i>ftsQ</i> gene	This work
pCI _{P22} - <i>ftsW</i>	pCI _{P22} derivative carrying the <i>ftsW</i> gene	This work
pCI _{P22} - <i>ftsW</i> _{1–226}	pCI _{P22} derivative carrying the <i>ftsW</i> gene fragment from 1 to 226 bp	This work
pCI _{P22} - <i>ftsW</i> _{1–933}	pCI _{P22} derivative carrying the <i>ftsW</i> gene fragment from 1 to 933 bp	This work
pCI _{P22} - <i>ftsW</i> _{1–534}	pCI _{P22} derivative carrying the <i>ftsW</i> gene fragment from 1 to 534 bp	This work
pCI ₄₃₄ - <i>ftsW</i>	pCI ₄₃₄ derivative carrying the <i>ftsW</i> gene	This work
pCI _{P22} -divIB	pCI _{P22} derivative carrying the <i>divIB</i> gene from <i>S. pneumoniae</i>	This work
pCI _{P22} - <i>ftsQ</i> Δ8	pCI _{P22} derivative carrying the <i>ftsQ</i> 147–171 bp deleted gene	This work
pTTQ18-GFP-FtsW	pTTQ18-GFP derivative carrying the <i>ftsW</i> gene	This work
pTTQ18-GFP-FtsN	pTTQ18-GFP derivative carrying the <i>ftsN</i> gene	This work
pTTQ18-GFP-FtsQ	pTTQ18-GFP derivative carrying the <i>ftsQ</i> gene	This work
pTTQ18-GFP-FtsK _{1–666}	pTTQ18-GFP derivative carrying the <i>ftsK</i> _{1–666} gene	This work
pTTQ18-GFP-FtsI	pTTQ18-GFP derivative carrying the <i>ftsI</i> gene	This work
pTTQ18-GFP-DivIB	pTTQ18-GFP derivative carrying the <i>divIB</i> gene	This work
pBAD33-GST-FtsQ	pBAD33-GST derivative carrying the <i>ftsQ</i> gene	This work
pBAD33-GST-FtsI	pBAD33-GST derivative carrying the <i>ftsI</i> gene	This work
pCI- <i>ftsQ</i> _{1–171}	pCI derivative carrying the <i>ftsQ</i> gene fragment from 1 to 171 bp	This work
pCI- <i>ftsQ</i>	pCI derivative carrying the <i>ftsQ</i> gene	This work

gene downstream of the GFP gene in the plasmid pTTQ18-GFP (V. D'Ulisse and others, unpublished). Analogously, the GST (glutathione S-transferase of *Schistosoma japonicum*) derivatives were obtained by cloning the gene of interest downstream of the GST gene in the plasmid pBad33-GST (V. D'Ulisse and others, unpublished).

Preparation of extracts for co-immunoprecipitation experiments. Cultures of *E. coli* DH5α harbouring the recombinant plasmids containing the genes of interest were fused in-frame to GST and GFP or to the λ repressor cI and GFP, respectively. Cultures were grown in LB supplemented with the appropriate antibiotics to OD₆₀₀ 0.3–0.4. The expression of the tagged genes was induced by

adding 1×10^{-4} M IPTG (for the GFP or cI-fused genes) and 0.2% arabinose (for the GST-fused gene) and the culture was grown for 4 h. The culture was then centrifuged, washed and resuspended (1/300, v/v) in lysis buffer (EDTA 1 mM pH 8, HEPES 25 mM pH 7.6, 0.1 mg lysozyme ml⁻¹) and cooled on ice for 30 min. Membrane fractions were then prepared as described by Buddelmeijer & Beckwith (2004). Soluble fraction was prepared by centrifuging the lysed sample at 50 000 g for 45 min and recovering the supernatant.

Co-immunoprecipitation studies. Immunoprecipitation experiments were similar to those described by Duong & Wickner (1997). Protein concentration was determined by the Bradford method.

Fig. 1. Schematic representation of pCl₂₂-ftsQ1Q2.

The beads (Protein A-Sepharose) were divided into aliquots of 50–100 µl in microcentrifuge tubes; to each tube, 10 µl GFP mouse monoclonal antibodies (1:100) (Santa Cruz Biotechnology) was added. The samples were incubated for 15–60 min at room temperature and gently mixed on a suitable shaker. After centrifugation, samples were washed with 1 ml washing buffer (20 mM HEPES buffer, pH 7.5, containing 150 mM NaCl, 0.1% Triton X-100 and 10%, v/v, glycerol), then 0.1–1 ml cell lysate was added to each tube.

Samples were incubated from 90 min to overnight at 4 °C, with gentle mixing on a suitable shaker. Immunoprecipitated complexes were collected by centrifugation at 3000 g for 2 min at 4 °C.

Western blotting. Electrophoresis and immunoblotting were performed as described by Laemmli (1970) and Towbin *et al.* (1979), respectively. The nitrocellulose membranes were probed with cI rabbit polyclonal antibodies (1:6000) or with GST mouse monoclonal antibodies (1:1000), depending on the specific cI- or GST-fused protein used in the experiment, and detected with ECF reagents (Amersham Pharmacia).

In some experiments, anti-GST antibodies (Santa Cruz Biotechnology) were used instead of anti-cI since the latter were no longer commercially available. Antiserum against FtsQ (MV J11), kindly provided by M. Vicente (CSIC, Madrid, Spain), was pre-adsorbed onto VIP210, according to Dopazo *et al.*, (1992), and then processed as described by Sherman & Goldberg (1992) for final steps of the purification.

Complementation of strain JOE170. Strain JOE170/pQ and its derivatives transformed with various plasmids were grown in LB medium supplemented with 0.2% L-arabinose and 50 µg ampicillin ml⁻¹. After overnight incubation at 37 °C, cells were centrifuged, and resuspended in SM buffer for 1 h. After this time of starvation, bacteria were plated on LB plates supplemented or not with arabinose in order to determine c.f.u. The efficiency of plating (e.o.p.) corresponds to the ratio between the bacterial titre on LB medium and that on LB medium supplemented with arabinose.

In the case of the growth kinetics, bacteria grown to OD₆₀₀ 0.2 in LB supplemented with arabinose were centrifuged, washed twice and resuspended in SM for 1 h. After starvation, bacterial cultures were diluted in prewarmed LB containing 1 × 10⁻⁴ M IPTG to a final concentration of about 1 × 10⁴ cells ml⁻¹ and grown at 37 °C. Over time, aliquots were withdrawn and plated on LB supplemented with arabinose.

RESULTS

The FtsQ protein domains specifically involved in interactions with proteins FtsB, FtsI, FtsL, FtsN and FtsW were identified by using the prokaryotic two-hybrid assay (THA)

called 'two-phages' (TP) developed in our laboratory (Di Lallo *et al.*, 2001). To study protein–protein interactions, we used the THA, because it is the easiest and quickest method, especially when the number of interactions to be tested is high (for a review see Legrain & Selig, 2000).

To date, interactions between the whole FtsQ and the other cell division partners have been confirmed by another THA, called BACTH (Karimova *et al.*, 2005), but only the interactions FtsQ–FtsL and FtsQ–FtsB were confirmed by biochemical experiments (Buddelmeijer & Beckwith, 2004). Therefore, to confirm and strengthen the TP and THA data (Di Lallo *et al.*, 2003; Karimova *et al.*, 2005) we analysed the interactions between FtsQ and the other partner proteins, namely FtsI, FtsN, FtsK and FtsW, by co-immunoprecipitation experiments.

Study of interactions between FtsQ and the other cell division partners by co-immunoprecipitation

Since we did not have at our disposal specific antibodies against these proteins, the assays were performed on the division proteins fused with tags (the λ repressor cI, GST or GFP), for which commercial antibodies are available. As a first step, we tested the specificity of these antibodies and our results showed that there was no cross-hybridization, i.e. the anti-cI, anti-GST and anti-GFP antibodies only recognize the cI-, GST- and GFP-fused protein, respectively (Fig. 2A, lanes 3 and 4; Fig. 2C, lane 1).

Fig. 2 shows the results of the FtsQ–FtsW, FtsQ–FtsN, FtsQ–FtsI and FtsQ–FtsK co-immunoprecipitation experiments. As expected from the TP and THA, in every case an interaction between the pairs of proteins was observed.

Identification of FtsQ interaction domains by means of the THA

To identify the FtsQ domains involved in the interactions with the other cell division proteins, we constructed recombinant plasmids harbouring partial deletions of the *ftsQ* gene encoding truncated forms of the protein and swap constructs, where the FtsQ cytoplasmic and/or MSS (membrane-spanning segment) domains were substituted with that of other proteins (Guzman *et al.*, 1997). These constructs were used in the prokaryotic THA (Di Lallo *et al.*, 2001). The rationale is that if two chimeric proteins formed by the N-terminal portion of phage 434 cI repressor fused in-frame with the division protein X (cI₄₃₄-X), and the N-terminal portion of phage P22 cI repressor fused in-frame with the division protein Y (cI_{P22}-Y), form the heterodimer cI₄₃₄-X/cI_{P22}-Y by interaction of their C-terminal domains, a fully functional repressor will be formed. This repressor will be able to shut down the expression of the *lacZ* reporter gene under the control of the 434/P22 hybrid promoter/operator region.

In our case, X is constituted by various fragments of the *ftsQ* gene to form the recombinant plasmids (pC₄₃₄-X), and Y by

Fig. 2. Co-immunoprecipitation experiments (Coip). (A) Protein extracts were immunoprecipitated with anti-GST (left) and anti-GFP (right) antibodies. Lanes 1 and 5, Coip of GFP-FtsW and GST-FtsQ detected with anti-GFP (lane 1) and anti-GST (lane 5) antibodies, respectively. Lanes 2 and 6, Coip of GFP-FtsN and GST-FtsQ detected with anti-GFP (lane 2) and anti-GST (lane 6) antibodies, respectively. Lane 3, Western blotting of GST-FtsQ detected with anti-GFP antibodies. Lane 4, Western blotting of GFP-FtsN detected with anti-GST antibodies. (B) Protein extracts were immunoprecipitated with anti-GST (top) and anti-GFP (bottom) antibodies. Lanes 1 and 5, Coip of GFP-FtsK and GST-FtsQ detected with anti-GFP (lane 1) and anti-GST (lane 5) antibodies, respectively. Lanes 2 and 6, Coip of GFP-DivIB and GST-FtsI detected with anti-GFP (lane 2) and anti-GST (lane 6) antibodies, respectively. Lanes 3 and 7, Coip of GFP-FtsQ and GST-FtsI detected with anti-GFP (lane 3) and anti-GST (lane 7) antibodies, respectively. Lane 8, Western blotting of GST-FtsQ detected with anti-GFP antibodies. Lane 4, Western blotting of GFP-FtsQ detected with anti-GST antibodies. Lane 9, Western blotting of GST-FtsQ detected with anti-GST antibodies. (C) Protein extracts were immunoprecipitated with anti-GFP. Lane 1, Western blotting of GST-FtsQ detected with anti-cl antibodies. Lane 2, Coip cl-FtsQ₁₋₅₇ and GFP-FtsQ detected with anti-cl antibodies. Lane 3, Coip of cl-FtsQ and GFP-FtsQ₁₋₅₇ detected with anti-cl antibodies. Lanes M, molecular mass markers.

the various division genes whose products interact with FtsQ, namely FtsB, FtsI, FtsK, FtsL, FtsN and FtsW, to form the recombinant plasmids (pcI_{P22}-Y). Pairs of recombinant plasmids encoding these chimeric repressors were co-transformed into the recipient strain R721 carrying the reporter gene *lacZ* under the control of the hybrid promoter/operator 434-P22. β -Galactosidase synthesis, which is constitutive in the strain without plasmids, is repressed in the presence of the two plasmids only if the two chimeric repressors interact.

Residual β -galactosidase activity was evaluated for each strain and compared to that of the parental strain without plasmids. Each value reported in Figs 3 and 4 is the mean of five independent determinations. The relevance of the values has been discussed previously (Di Lallo *et al.*, 2003). Standard deviations (not reported) were of the order

of 5 % compared to the mean values of the β -galactosidase activities obtained in the various determinations. In this paper, we assumed that residual β -galactosidase synthesis under 50 % is indicative of repression and hence of protein interaction, whereas for synthesis above 50 %, the interaction is uncertain or negligible. The same results were obtained when the genes encoding the proteins under investigation were cloned in the reciprocal vectors, i. e. cI₄₃₄-X/cI_{P22}-Y and cI₄₃₄-Y/cI_{P22}-X (data not shown).

Role of the cytoplasmic, transmembrane and periplasmic FtsQ domains in protein-protein interactions

Truncated forms of FtsQ and swap constructs (Guzman *et al.*, 1997) were used to study the interaction of this protein

Fig. 3. (A) Schematic representation of *E. coli* FtsQ protein showing the DNA domains corresponding to the cytoplasmic (Cyt.), the membrane-spanning (MSS) and the periplasmic domains. (B) Various *ftsQ* fragments (left) were cloned in both *pcl*₂₂ and *pcl*₄₃₄ and tested for their ability to interact with the other Fts proteins by the TP THA (Di Lallo *et al.*, 2003). Residual β -galactosidase activity of less than 50 % (in bold) indicates an interaction between the Fts protein and the peptide coded by the particular FtsQ fragment (right). nt, Not tested.

with itself and the other cell division proteins. The results, reported in Figs 3 and 4, show that: (i) the cytoplasmic domain deletion does not affect the ability of FtsQ to interact with itself and FtsN, FtsI, FtsL, FtsB or FtsW (Fig. 3, row c) but results in loss of FtsK interaction; (ii) no interaction is observed when both the cytoplasmic and transmembrane domains are deleted (Fig. 3, row b), although this truncated protein is normally produced, as shown by Western blot experiments performed with anti-FtsQ antibodies (data not shown); (iii) all these interactions, again with the exception of FtsK, are restored with the swap construct in which the FtsQ MSS domain is substituted with that of a protein involved in a process totally unrelated to cell division such as the membrane protein MalF, which is required for maltose transport in *E. coli* (Froshauer & Beckwith, 1984; Hofnung, 1974) (Fig. 4, row a), (iv) the presence of a cytoplasmic domain is essential for FtsK interaction, but the domain itself is not specific since the FtsQ cytoplasmic domain can be substituted with that of FtsN and the interaction is conserved (Fig. 4, row c). It is important to note that FtsN does not interact with FtsK (Di Lallo *et al.*, 2003). Moreover, since it has been reported that FtsN can interact with FtsA (Corbin *et al.*,

2004) we studied the interactions of the swap construct NNQ with FtsA. As shown in Fig. 4(A), no FtsA interaction was evident (rows c, d, e) whereas normal interactions were observed with FtsQ, FtsN, FtsI, FtsK and FtsB (rows c and d).

From these results we can conclude that all the interactions of FtsQ both with itself and with the other cell division proteins are localized in the periplasmic domain of the protein although the MSS domain is necessary.

Overall, all these results agree with the conclusions of Dai *et al.* (1996) showing that the FtsQ periplasmic domain is necessary for the division process. In addition, constructs in which the cytoplasmic domain was intact but the FtsQ MSS was replaced were still functional. Even the construct in which the MalF MSS replaced the FtsQ MSS complemented the null mutation. Thus, the specific sequence of the MSS of FtsQ is not required for its function and may act only as a membrane-anchoring sequence (Guzman *et al.*, 1997). Lastly, the need of the MSS domain for FtsQ interactions is in agreement with the fact that this protein is post-translationally translocated (Scotti *et al.*, 1999) and is not able to fold correctly in the cytoplasm.

Fig. 4. (A) Domain-swapping experiments. Various DNA constructs encoding proteins (left), described in Methods, were cloned in both *pcl₂₂* and *pcl₄₃₄* and tested for their ability to interact with the other Fts proteins by the TP THA (Di Lallo *et al.*, 2003). Residual β -galactosidase activity of less than 50 % (in bold) indicates an interaction between the Fts protein and the peptide coded by the particular construct (right). (B) Interaction between FtsQ₂₁₋₂₀₂ and FtsW fragments. Residual β -galactosidase activity of less than 50 % indicates an interaction between the two peptides under investigation. nt, Not tested.

Identification of the FtsQ periplasmic subdomains involved in interactions with FtsB, FtsI, FtsL, FtsN, FtsK and FtsW

FtsQ interaction with itself and other bitopic proteins, FtsB, FtsI, FtsL, FtsN. Progressive deletions of the FtsQ periplasmic domain indicate that the last 43 aa are essential for FtsL interaction. Indeed, the residual β -galactosidase activity of 23 %, suggesting an interaction between FtsQ and FtsL, increased to 77 % when the last 43 residues of FtsQ were deleted (Fig. 3, rows c and d), in agreement with the results of Karimova *et al.* (2005). Analogously, the interaction of FtsB can be localized between residues 136 and 202 (Fig. 3, rows e and f).

These data are also consistent with the observation of Chen *et al.* (2002) that an ochre mutation interfering with translation of the last 29 aa is lethal and with the hypothesis proposed by Buddelmeijer *et al.* (1998) that the C-terminus of FtsQ might define a part of the protein involved in the interactions with FtsL and FtsB. This same region shows up as surface exposed in structure predictions for FtsQ, consistent with its possible role in protein-protein interactions.

Surprisingly, the interaction of FtsQ with itself and with FtsN and FtsI seems to be restricted to 8 aa. As shown in Fig. 3, progressive deletions of the *ftsQ* sequence from the

C-terminus to residue 57 still maintain the interaction ability, which is simultaneously lost for all three proteins with the fragment containing residues 1–49. The interaction between the whole FtsQ and the FtsQ fragment 1–57 was also confirmed by co-immunoprecipitation experiments using the GFP-FtsQ and the cI-FtsQ₁₋₅₇ fusion proteins as described above. The results reported in Fig. 2(C) are in agreement with those obtained with the THA. To elucidate whether other sites of FtsQ were involved in the interaction of the protein with itself, FtsN and FtsI, we constructed a mutated FtsQ protein (FtsQ8D) in which these 8 aa were deleted and replaced by 2 aa derived by religation of the *Xba*I site, as described in Methods. This deletion mutant retained the interaction ability of the wild-type FtsQ protein (Fig. 3, row m), whereas the FtsQ fragment 1–72 containing the 8D mutation was unable to interact with the FtsQ partners (Fig. 3, row o). Also in this case, the production of the truncated protein was verified by Western blot experiments with anti-FtsQ antibodies (data not shown). Taken together, these two results indicate that the 8 aa are effectively involved since their deletion affects the interaction ability, but also suggest that at least two sites are involved in interaction with FtsQ, FtsN and FtsI. The first site is located in the N-terminal part of the periplasmic domain between residues 49 and 57, and the second is located between residues 202 and 234 (Fig. 4, rows e and d), although Karimova *et al.* (2005) observed that a deletion of

Table 3. Efficiency of plating in the presence and absence of arabinose of *E. coli* strain JOE170/pQ and its derivatives

The values are the mean of three independent experiments.

Strain	Efficiency of plating (–Ara/ + Ara)
JOE170/pQ	1.2×10^{-6}
JOE170/pQ/pcl _{P22} -ftsQ	1
JOE170/pQ/pcl _{P22} -ftsQ1Q2	2.7×10^{-3}
JOE170/pQ/pcl _{P22} -ftsQD8	3.3×10^{-2}
JOE170/pQ/pcl _{P22} -divIB	$\sim 1 \times 10^{-1}$

the last 30 aa of FtsQ (FtsQ_{1–246}) abolished the capacity of the protein to dimerize as well to associate with all tested proteins. The fact that FtsB but not FtsA is able to interact with both the constructs indicates that in both cases the truncated proteins are produced and the FtsN cytoplasmic and transmembrane fragments present do not interfere with the results obtained.

In order to analyse the physiological role of the FtsQ region between residues 49 and 57, we transformed the plasmid carrying the FtsQ8D mutant under *plac* control into strain JOE170/pQ and studied its ability to complement an *ftsQ* null mutant in conditions of depletion of the wild-type FtsQ protein carried by the pQ plasmid. The e.o.p. of strain JOE170/pQ, which is 1 in the presence of arabinose (inducer of the expression of the wild-type *ftsQ* gene), was about 1.2×10^{-6} without arabinose (Table 3). When this strain,

harbouring the plasmid carrying the FtsQ8D mutant, was plated in the presence of IPTG but without arabinose, the e.o.p. was 3.3×10^{-2} (mean of three independent experiments). This reduction of about 100-fold suggests that the region between residues 49 and 57 participates in FtsQ functionality but is not essential. This result was also confirmed by the study of the kinetics of growth of strain JOE170/pQ, harbouring the plasmid carrying the FtsQ8D mutant, in the absence of arabinose (Fig. 5). The generation time of this strain is in the order of 60 min, i.e. about double that observed upon expression of the wild-type *ftsQ* gene.

FtsQ interaction with the polytopic proteins FtsK and FtsW. The interaction site between FtsQ and FtsK was also localized in the periplasmic fragment between residues 202 and 234; interestingly, as mentioned above, this interaction requires the presence of a cytoplasmic domain, though not necessarily that of FtsQ. In fact, the localization of FtsK interaction was studied in the presence of the FtsN cytoplasmic domain (Fig. 4, rows c, d, e).

Fig. 3 shows that the FtsQ fragment 1–136 is able to interact with FtsW. This fragment includes the region called POTRA that is a conserved domain in the FtsQ family and a class of β -barrel outer-membrane proteins (Sanchez-Pulido *et al.*, 2003) identified in the α -domain of DivIB/FtsQ (Robson & King, 2006). The fact that a deletion of the POTRA region results in loss of interaction (fragment 1–105) suggests that this domain could have a role in FtsW interaction.

Fig. 5. Kinetics of growth of strain JOE170/pQ and its derivatives. The experiments were performed as described in Methods. (A) Growth of JOE170/pQ in the presence (■) and absence (○) of arabinose. (B) Growth of JOE170/pQ (●) and its derivative carrying plasmid pcl₂₂ftsQD8 (○) in the absence of arabinose. (C) Growth of JOE170/pQ (○) and its derivatives carrying plasmid pcl₂₂Q1Q2 (●), pcl₂₂Q1 (▲) or pcl₂₂Q2 (△) in the absence of arabinose. (D) Growth of JOE170/pQ (○) and its derivative carrying plasmid pcl₂₂DivIB (●) in the absence of arabinose.

Since the FtsQ periplasmic domain is essential for FtsW interaction, one could imagine that, on the other hand, the FtsW periplasmic spans are involved too. For this reason, we constructed two FtsW deletions, determined on the basis of the predicted topology model of the *E. coli* protein (Gerard *et al.*, 2002; Pastoret *et al.*, 2004), containing two spans (residues 1–181, containing spans 67–75 and 130–140) and only one span (residues 1–75, containing the span 67–75), respectively. The ability of these fragments to interact with FtsQ was then studied. As shown in Fig. 4(B), the smallest FtsW fragment able to interact with FtsQ is that containing just the first span.

Is it possible to divide the FtsQ periplasmic domain into functional subdomains?

It is possible to assume that the FtsQ domain architecture is similar to that of the orthologous protein DivIB of the Gram-positive thermophile *Geobacillus stearothermophilus*, whose 3D structure was recently resolved (Robson & King 2006). The extracytoplasmic region of DivIB comprises three discrete domains: α (residues 47–116), β (residues 117–230) and γ (residues 231–261) from the membrane-proximal N-terminus to the C-terminus. The α - and β -domains are structurally autonomous whereas the γ -domain is proteolytically sensitive and presumably unstructured in the absence of other divisomal proteins.

In this section the term ‘domains’ will be used to identify the FtsQ periplasmic regions involved in protein–protein interactions, which will thereafter be compared with that deriving from the 3D structure of DivIB/FtsQ.

According to the above results, various sites of the FtsQ periplasmic domain are involved in its interactions with other division proteins. In addition, from the point of view of protein–protein interaction, this domain can be subdivided into three parts: (i) proximal to the transmembrane segment, residues 49–136, specific for FtsQ, FtsN, FtsI and FtsW; (ii) central, residues 136–202, where the FtsB interaction is localized; and (iii) C-terminal, residues 202–277, for FtsK, FtsL and for FtsQ, FtsN and FtsI (Fig. 6).

These observations raised the question whether the simultaneous presence of independent peptides corresponding to these fragments could complement the FtsQ depletion. To simplify the experiment, the complementation of the null FtsQ mutant, JOE170/pQ, was performed with two fragments which reconstitute the whole protein

Fig. 6. Schematic representation of *E. coli* FtsQ protein showing the DNA domains corresponding to the periplasmic domains involved in interaction with the other Fts proteins.

sequence (Fig. 1): the first fragment (called Q1) extends from residues 1 to 136, involved in FtsQ, FtsI, FtsN and FtsW interactions, and the second (called Q2) extends from 136 to 277, in which are localized the FtsB interaction site and the second site for FtsQ, FtsI, FtsN and FtsL interactions. Upstream of the coding sequence of Q2, the FtsN cytoplasmic and MSS domains were inserted, as described in Methods, to allow the peptides to anchor to the cell membrane, avoiding the possibility of recombination with the analogous domains of FtsQ, which could rearrange the plasmid sequence. These two domains do not interact with the other division proteins (Fig. 4, row f). If complementation occurs, the biological activity of this construct is verified.

Complementation experiments were performed as described in Methods. Aliquots of bacterial cultures of strain JOE170/pQ harbouring pC_{IP22}-ftsQ1Q2 grown in LB medium in the presence of arabinose to about 2×10^8 cells ml⁻¹ were centrifuged, washed and resuspended in medium without arabinose. After 1 h, Q1Q2 expression was induced by addition of 1×10^{-4} M IPTG, and, over time, aliquots were plated on arabinose-supplemented medium. As shown in Fig. 5(C), during the first 3 h, the strain harbouring pC_{IP22}-ftsQ1Q2 grew with a generation time of about 40 min (i.e. similar to that of the parental strain in the presence of arabinose), whereas JOE170/pQ without the plasmid was unable to grow. The Q1 and Q2 fragments, cloned separately on the same vectors, showed a different behaviour: Q1 allowed transient bacterial growth, with a generation time of about 70 min for the first 2 h, whereas JOE170/pQ cells containing Q2 alone died with a kinetics comparable with that of the parental strain without plasmid.

The above observations were confirmed by the results of the experiments in which the e.o.p. of the strains in the presence and in absence of arabinose in the medium was evaluated (Table 3). The value of 1.2×10^{-6} shown by JOE170/pQ increased to 2.7×10^{-3} in the presence of plasmid pC_{IP22}-ftsQ1Q2. Lastly, to exclude the possibility that the complementation could be due to the presence of a residual number of wild-type FtsQ molecules able to form multimers with the truncated form of the protein, we evaluated the amount of native FtsQ present in depleted cells. Western blot analysis of bacterial extracts obtained in the same conditions as used for complementation experiments showed that FtsQ was absent after 1 h of depletion (Fig. 7).

Taken together, these results suggest that, in order to complement, the two peptides Q1 and Q2 co-localize at the Z-ring level and interact with the other division proteins. Compared to the behaviour of Q1Q2, Q1 alone is still able to work, at least partially, whereas Q2 has negligible activity, although this fragment carries the interaction sites for the FtsQ division partners FtsB, FtsK and FtsL as well as the second site for FtsQ itself, FtsI and FtsN.

Fig. 7. Analysis by Western blotting of the amount of FtsQ in strain JOE170/pQ in the absence of arabinose. Strain JOE170/pQ was grown in LB in the presence of arabinose. At OD₆₀₀ 0.3 an aliquot was withdrawn and the remaining culture was processed as described in Methods. Protein extracts were then analysed by Western blotting with anti-FtsQ antibodies. Lane 1, in the presence of arabinose ($t=0$ of the experiment); lane 2, after 1 h starvation in SM buffer without arabinose; lanes 3 and 4, after resuspension in LB without arabinose ($t=2$ h and $t=3$ h of the experiment, respectively). The same experiment was repeated with a JOE170/pQ derivative carrying plasmid *pcl*₂₂DivIB. Lane 5, in the presence of arabinose ($t=0$ of the experiment); lane 6, after 1 h starvation in saline without arabinose; lane 7: after resuspension in LB without arabinose ($t=3$ h of the experiment). Lanes M, molecular mass markers.

Complementation of an *ftsQ* null mutant with the orthologue DivIB of *S. pneumoniae*

It has been shown that FtsQ forms an *in vivo* complex with FtsL and FtsB (Buddelmeijer & Beckwith, 2004). Since the *S. pneumoniae* DivIB (FtsQ) shows the same properties, forming a trimer with the *S. pneumoniae* orthologous proteins FtsL and DivIC (FtsB) (Noirclerc-Savoye *et al.*, 2005), we asked whether DivIB could interact with the *E. coli* proteins involved in this trimer formation. In other words, whether the interacting domains were somehow conserved during evolution, supporting the hypothesis of a ‘minimal common divisome’ whose specific characteristics were thereafter differentiated by evolution.

Results of interaction experiments between DivIB and the *E. coli* FtsQ partners, and of interactions between FtsQ and *S. pneumoniae* FtsL and DivIC (FtsB), are reported in Table 4. As far as the trimer formation is concerned, only the interaction DivIB–FtsQ is conserved. In addition, DivIB was able to interact with the *E. coli* FtsI protein. These results were confirmed by the co-immunoprecipitation experiments reported in Fig. 2(B). Surprisingly, in spite of its inability to interact with most of the FtsQ partners, DivIB was able to sustain, at least partially, the growth of the *E. coli* *ftsQ* null mutant (Fig. 5): the wild-type *divIB* gene, cloned in a plasmid under *plac* promoter control, was inserted by

Table 4. Interaction between the *S. pneumoniae* DivIB protein and the *E. coli* Fts proteins

The assay was performed as described in Methods. Residual β -galactosidase activity of less than 50% indicates an interaction between the two proteins under investigation.

	Residual β -galactosidase activity (%)								
	FtsZ	FtsA	FtsB	FtsN	FtsK	FtsL	FtsW	FtsQ	FtsI
DivIB	63	98	85	95	65	100	100	23	29

transformation into strain JOE170/pQ and its ability to sustain the growth of the strain in conditions of FtsQ depletion was tested. The e.o.p. of strain JOE170/pQ with and without arabinose in the medium, which is of the order of 10^{-6} , clearly increases in the presence of DivIB and in an IPTG concentration-dependent manner (data not shown), reaching about 10^{-1} with 1×10^{-3} M IPTG (Table 3). In the absence of arabinose, the colonies were smaller (diameter about 2–3 times less) than those grown with arabinose. In addition, this rescue was also observed in broth, where the filamentous phenotype of strain JOE170/pQ, observed after 3 h of growth without arabinose, was lost in the presence of the DivIB plasmid. Indeed, in this case the bacteria were heterogeneous in size, with cells of normal size and cells showing some filamentation (data not shown).

A possible way to explain this complementation could be that the presence of the *S. pneumoniae* protein may somehow stabilize the *E. coli* FtsQ protein, protecting the cell from death. This is not the case since, as shown by the Western blot experiments (Fig. 7), the stability of FtsQ is the same in the absence and in the presence of DivIB. The observed complementation suggests that DivIB could localize at the *E. coli* Z-ring level without interacting with the other proteins that normally interact with FtsQ.

DISCUSSION

In *E. coli*, a multiprotein complex of about 10 different proteins, generally conserved amongst prokaryotes, forms the division machinery (for reviews, see Margolin, 2000; Weiss, 2004; Vicente *et al.*, 2006). Even if all of these proteins are essential, the role and biochemical activities of many of them remain to be discovered. It is known that some of them (FtsB, FtsQ, FtsL, FtsN, FtsI) are bitopic and some (FtsK and FtsW) are polytopic membrane proteins.

Genetic data, such as suppression of thermosensitive mutants in cell division proteins by means of overexpression of another division protein (Dai *et al.*, 1993, 1996; Draper *et al.*, 1998), as well as two-hybrid screens (Di Lallo *et al.*, 2003; Karimova *et al.*, 2005) and biochemical techniques (Buddelmeijer & Beckwith, 2004) indicate that these proteins have a complex web of interaction. FtsQ is a well-documented example. By a bacterial THA, it was suggested that FtsQ interacts with FtsI, FtsL and FtsN (Di

Lallo *et al.*, 2003). This conclusion was recently confirmed by Karimova *et al.* (2005) using a different THA. In addition, in each case by THA, it was shown that FtsQ interacts with FtsK and FtsW (Di Lallo *et al.*, 2003) and FtsB (this work). Interactions between FtsQ, FtsL and FtsB were also confirmed by biochemical methods (Buddelmeijer & Beckwith, 2004).

Two aims formed the basis of this work: (i) to determine the FtsQ domains involved in interactions with the other division proteins; and (ii) to investigate the physiological role of the interactions described between FtsQ and its cell division partners, i.e. are all the identified interactions essential for septum construction?

FtsQ domains involved in the interactions with other division proteins

The structure of DivIB, an FtsQ orthologue (Robson & King, 2006), predicts that the extracytoplasmic region of DivIB comprises three discrete domains: α (residues 47–116), β (residues 117–230) and γ (residues 231–261) from the membrane-proximal N-terminus to the C-terminus. The α - and β -domains are structurally autonomous whereas the γ -domain is proteolytically sensitive and presumably unstructured in the absence of other divisomal proteins.

The architecture of FtsQ is expected to be very similar based on sequence homology with DivIB. The study of the progressive deletions in the *ftsQ* gene and domain-swap constructs suggests that the FtsQ self-interaction as well as its interactions with the other cell division proteins are localized in the periplasmic domain of the protein, in which it is possible to identify three regions comparable to that described by the 3D structure of DivIB (Fig. 6). However, from data presented in Figs 3 and 4, six interaction subdomains rather than three can be identified. Domain I (residues 1–21) contains the FtsK interaction site; domain II (residues 21–57) contains one of the two sites involved in FtsQ, FtsI and FtsN interactions; domain III (residues 105–136) is responsible for the FtsW interaction. In domain IV (residues 136–202), the FtsB interaction is localized, while in domain V (residues 202–234) the FtsK interaction site is located together with that of FtsQ, FtsI and FtsN. Lastly, in domain VI (residues 234–277) we find the interaction site for FtsL.

This suggests that within the structured regions α , β and γ found in DivIB smaller subdomains are probably involved in specific interactions.

Interestingly, two protein fragments are implicated in FtsQ, FtsI and FtsN interactions for which each of these sites is sufficient, since the FtsQ mutants carrying the fragment spanning between residues 1–57 as well as that between 137 and 234 are still able to interact with FtsQ itself, FtsI and FtsN. The role of residues 49–57 was also proved by biochemical experiments showing co-immunoprecipitation of the FtsQ fragment 1–57 with both FtsI and FtsQ. The

importance of this region is also deduced by the observation that the strain harbouring a recombinant plasmid with the FtsQ protein deleted for residues 49–57 showed a 100-fold reduction in its ability to complement a bacterial strain depleted for FtsQ compared to the strain with the plasmid encoding the wild-type protein.

The analysis of the distribution of FtsQ sites of interactions with the other division proteins raises the hypothesis of its having a modular structure. We asked whether the subdomains described above could have a physiological role by testing if cells expressing peptides corresponding to these subdomains could complement the FtsQ depletion. This complementation was studied with two fragments. The first, called Q1, contains residues 1–136 and the second, Q2, spans from 136 to 277.

Our results indicate that the presence of plasmid Q1Q2 transiently complements the FtsQ depletion in strain JOE170/pQ, suggesting that the two peptides are able to localize independently at the Z-ring level and interact with the other division partnerships. The different behaviour shown by the two fragments as far as the complementation ability is concerned could be explained by a different turnover rate of the two peptides.

Robson & King (2006) proposed that the α -domain corresponds to the POTRA domain that was predicted on the basis of bioinformatic analyses to be present in DivIB/FtsQ and exists as an autonomously folded protein domain. It was suggested that the POTRA domain might function as a chaperone that specifically recognizes secretion or assembly-competent forms of polypeptides (Sanchez-Pulido *et al.*, 2003). In addition, the α -domain could serve as a chaperone for its cognate divisomal partner FtsL. DivIB forms a ternary complex with FtsL and DivIC (Goehring & Beckwith, 2005; Buddelmeijer & Beckwith, 2004; Noirclerc-Savoye *et al.*, 2005). These latter two proteins are unstructured and intrinsically unstable *in vitro* in the absence of DivIB (Robson & King, 2006), and FtsL is rapidly degraded in a *divIB* null strain at the non-permissive temperature (Daniel & Errington, 2000).

From these elements, we can deduce that Q1 roughly corresponds to the α -domain and Q2 to domains β and γ . According to the model of Robson & King (2006), Q1, besides its direct interaction with the other proteins, could also act as a chaperone. This activity could protect FtsL and/or other proteins from degradation. The Q2 fragment alone, without this chaperone Q1 activity, is therefore unable to complement since it cannot prevent the protein degradation. Future work should address this hypothesis.

Heterologous complementation: a new approach for *in vivo* studies of protein–protein interaction

One of the main problems in studying protein–protein interactions, identified by both *in vivo* genetic and *in vitro*

biochemical methods, is to understand their biological role. It is possible that when many proteins form a complex, only a few interactions are essential. The role of the others may be to stabilize the structure in which they take part. In addition, it is possible that some interactions are only due to the protein's proximity to the complex without either determining its formation or having a role in it.

An approach to this problem could be to determine if it is possible *in vivo* to substitute the single components of a multiprotein complex with orthologous proteins from other bacteria, while retaining cell viability. In this case, the orthologous protein should be able to interact with the other proteins of the complex. In fact, it could be hypothesized that if a protein is conserved, as orthologue, the interactions with the partner proteins should also be conserved in the course of evolution. The loss of some interactions would thus suggest their 'secondary' role.

We showed that the *S. pneumoniae* DivIB can functionally substitute, at least partially, for the orthologous FtsQ protein of *E. coli*, although it interacts only with FtsQ and FtsI and not with the other FtsQ partners. This result was unexpected for two reasons. The first is that, although interspecies interactions amongst division proteins, such as FtsA and FtsZ, have been observed (Ma *et al.*, 1997), these occur when the species are quite closely related and this is not the case for *E. coli* and *S. pneumoniae*. The second is that, although both FtsQ and DivIB form a trimer with FtsB (DivIC in the case of *S. pneumoniae*) and FtsL, DivIB is unable to interact with the *E. coli* orthologue, in spite of its ability to suppress the FtsQ depletion.

The finding that an *E. coli* *ftsQ* null mutant can be complemented by *S. pneumoniae* DivIB suggests a possible new strategy for investigating the biological significance of the protein–protein interactions identified by the various methods. We can therefore argue that only the homodimeric and the FtsI interactions are essential. Interactions with FtsN, FtsK, FtsB, FtsL and FtsW could be only secondary and contribute to stabilizing the complex.

Another possible explanation could be that the local FtsQ competes with DivIB for the interactions. Only the FtsI–DivIB interaction could be strong enough to be seen. In contrast, in the complementation experiment, the local FtsQ is absent and DivIB may well interact with more partners. This hypothesis can be eliminated since it is well known that the copy number of native FtsQ coded by the bacterial chromosome is low whereas the number of FtsI and FtsN copies is high, due to the plasmid localization of the genes under investigation. In addition the same result is obtained when studying the interaction between FtsQ and the *S. pneumoniae* division proteins.

Lastly, there is also the possibility that the *S. pneumoniae* protein could be degraded in *E. coli*. Also this hypothesis can be ruled out since we have shown that the *S. pneumoniae*

division proteins normally interact with DivIB in *E. coli* (V. D'Ulisse and others, unpublished).

A function for FtsQ

FtsQ is arguably the most enigmatic divisomal protein. Discovered 25 years ago, its role in bacterial cell division remains unclear. It has been proposed that FtsQ provides a structural link between early- and late-assembling divisomal components (Di Lallo *et al.*, 2003). A function in regulation of divisome assembly was suggested by Piette *et al.* (2004) and deduced by the fact that this assembly is very sensitive to FtsQ overexpression in the presence of thermosensitive *ftsZ*, *ftsA* or *ftsI* mutations (Dai & Lutkenhaus, 1992). In addition, recent evidence suggests that the FtsQ orthologue DivIB in *Bacillus subtilis* might also play a role in linking chromosome segregation to asymmetric cell division during sporulation (Real *et al.*, 2005).

The finding that DivIB/FtsQ belong to a class of β -barrel outer-membrane proteins involved in transport or assembly of polypeptides (Sanchez-Pulido *et al.*, 2003) provided new elements to hypothesize a new function for FtsQ. Robson & King (2006) suggested that the POTRA domain might function as a chaperone that specifically recognizes secretion or assembly-competent forms of these polypeptides. These facts allow one to hypothesize a role for FtsQ of control of correct divisome assembly. Our data agree with both the scaffold and the assembly control role of FtsQ. (i) FtsQ interacts with at least six division proteins, but many of these interactions may not be essential, as suggested by the heterologous interactions between *S. pneumoniae* DivIB and the *E. coli* FtsQ interacting proteins; (ii) the whole FtsQ protein does not seem necessary for its biological activity, since the protein divided into two fragments is also able to partially sustain the growth of an *ftsQ* null mutant; and (iii) such complementation is also observable with the orthologous DivIB protein, which shows only 16 % homology with FtsQ (Massidda *et al.*, 1998).

These data could indicate that FtsQ behaves like the central element of a puzzle. In such a complex, the central element should also be able to work when broken in two parts, since each part could be kept bound to the other by its joining with the other pieces, i.e. the various proteins interacting with FtsQ. Moreover, the complementation experiments, which showed that Q1 alone works much like Q1Q2 whereas Q2 is only partially active, accord with the FtsQ chaperone role in the divisome assembly control.

In conclusion, from this paper the idea arises that the complementation studies of null mutants with orthologous proteins (as for the complementation of an *E. coli* *ftsQ* null mutant with the *S. pneumoniae* DivIB protein, described here) could represent a new strategy for investigating the biological significance of the interactions identified with current methods for studying protein–protein interactions. With this strategy it should be possible to distinguish

between the essential (or 'primary') and the dispensable (or 'secondary') interactions.

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