Central Domain of DivIB Caps the C-terminal Regions of the FtsL/DivIC Coiled-coil Rod*§

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DivIB(FtsQ), FtsL, and DivIC(FtsB) are enigmatic membrane proteins that are central to the process of bacterial cell division. DivIB(FtsQ) is dispensable in specific conditions in some species, and appears to be absent in other bacterial species. The presence of FtsL and DivIC(FtsB) appears to be conserved despite very low sequence conservation. The three proteins form a complex at the division site, FtsL and DivIC(FtsB) being associated through their extracellular coiled-coil region. We report here structural investigations by NMR, small-angle neutron and x-ray scattering, and interaction studies by surface plasmon resonance, of the complex of DivIB, FtsL, and DivIC from Streptococcus pneumoniae, using soluble truncated forms of the proteins. We found that one side of the “bean”-shaped central β-domain of DivIB interacts with the C-terminal regions of the dimer of FtsL and DivIC. This finding is corroborated by sequence comparisons across bacterial genomes. Indeed, DivIB is absent from species with shorter FtsL and DivIC proteins that have an extracellular domain consisting only of the coiled-coil segment without C-terminal conserved regions (Campylobacteriaceae). We propose that the main role of the interaction of DivIB with FtsL and DivIC is to help the formation, or to stabilize, the coiled-coil of the latter proteins. The coiled-coil of FtsL and DivIC, itself or with transmembrane regions, could be free to interact with other partners.

Cell division is one of the defining features of life. Understanding the division of bacteria is also required to find novel antibiotic strategies. Numerous studies, carried out mostly with the model organisms Escherichia coli and Bacillus subtilis have uncovered several components of the divisome, which can be defined as the ensemble of proteins localized at the division site and participating in the process. Comparison of genomes and deletion studies indicate that the core of the divisome comprises eight conserved, mostly essential proteins: FtsZ, FtsA, FtsK, FtsQ(DivIB), FtsL, FtsB(DivIC), FtsW, and FtsI.

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nomenclature applies to Gram-negative organisms, whereas Div nomenclature applies to Gram-positive bacteria. These proteins are listed here in the conditional order of their recruitment to the division site of E. coli (1–4).

Processes in which they participate have been attributed to several division proteins. FtsZ forms polymers with an annular distribution on the cytoplasmic side of the membrane and governs the recruitment of the other proteins. FtsA may mediate the interaction of FtsZ with the membrane. FtsK participates to the resolution of chromosome dimers, and possibly to the membrane fission. FtsL, and likely FtsW, participate to septal cell wall formation (1–4). In contrast, the roles of FtsQ(DivIB), FtsL, and FtsB(DivIC) have not been firmly linked to any particular process.

FtsQ(DivIB), FtsL, and FtsB(DivIC) are positioned in the middle of the conditional order of recruitment in E. coli and B. subtilis. When the temporality of the recruitment was examined, FtsQ(DivIB) was found to belong to the late recruits, together with the proteins involved in cell wall assembly (5). In E. coli, the presence of FtsL and FtsB at the division site is mutually dependent, and their localization depends on that of FtsQ (6, 7). In B. subtilis, the presence of FtsL and DivIC at mid-cell depends on that of DivIB, at the temperature at which DivIB is essential, and reciprocally (8, 9). A complex comprising FtsQ, FtsL, and FtsB was isolated from E. coli by co-immunoprecipitation (10), and reconstituted in vitro with recombinant soluble forms of pneumococcal DivIB, FtsL, and DivIC (11). The interaction of the three proteins was also confirmed by yeast and bacterial triple hybrid (12, 13).

The genes ftsL and ftsB(divIC) are essential in E. coli and B. subtilis (6, 14–16) and presumably in Streptococcus pneumoniae (17). The essentiality of ftsQ(divIB) in laboratory conditions varies between species. The gene ftsQ is essential in E. coli (18), but divIB is essential only at high temperatures in B. subtilis (9, 19), or in a chemically defined medium in S. pneumoniae (17). Under these conditions, the essentiality of DivIB appears to be a consequence of the protection from proteolysis that it provides to FtsL (8, 17).

FtsQ(DivIB), FtsL, and FtsB(DivIC) are bitopic membrane proteins with an N-terminal cytoplasmic region, a single transmembrane segment, and an extracytoplasmic region. The extracellular part is necessary and sufficient for the localization and function of FtsQ(DivIB), provided that it is anchored to the membrane (e.g. Refs. 20 and 21), although the transmembrane
segment also contributes to the localization (22, 23). The extracellular part is organized in three regions termed \( \alpha \), \( \beta \), and \( \gamma \). The crystal structure of a region consisting of the \( \alpha \)- and \( \beta \)-domains was solved for FtsQ from \textit{E. coli} and \textit{Yersinia enterocolitica} (24). The \( \alpha \)-domain, comprising about 70 amino acids proximal to the cytoplasmic membrane, corresponds to the POTRA (for polypeptide transport-associated) domain first identified by sequence analysis and proposed to function as a molecular chaperone (25). The \( \alpha \) and \( \beta \)-domains form the conserved region of the FtsQ(DivIB) protein. The \( \gamma \)-region constitutes a C-terminal tail. It is highly variable in length and sequence and predicted to be unfolded. The \( \gamma \)-region was not observed in the structures from \textit{E. coli} and \textit{Y. enterocolitica}, thus confirming its flexible nature (24).

The \( \alpha \)-domain in the recombinant soluble form of the extracellular part of DivIB from \textit{Geobacillus stearothermophilus} was digested by trypsin and therefore considered to be largely unfolded (26). The \( \gamma \)-region was also removed by trypsin digestion, together with a C-terminal fragment of the \( \beta \)-domain. The structure of the resulting shorter \( \beta \)-domain from \textit{G. stearothermophilus} was solved by NMR (26) and lacks the two C-terminal \( \beta \)-strands.

Localization epitopes have been identified in the transmembrane segment, the \( \alpha \)-domain, and a region encompassing the C-terminal part of the \( \beta \)-domain and \( \gamma \)-tail of DivIB from \textit{B. subtilis} (23). Likewise in \textit{E. coli}, a region in the \( \alpha \)-domain is required for localization of FtsQ, whereas the C-terminal region of the \( \beta \)-domain and the last \( \alpha \)-helix are required for recruitment of FtsL and FtsB (24). In \textit{S. pneumoniae}, the essentiality of DivIB in defined medium was found to reside in the C-terminal region of the \( \beta \)-domain (17).

No experimental structure is known for FtsL or FtsB(DivIC). Both are small proteins comprising between 90 and 140 amino acids. The number of residues is sometimes larger, as in \textit{Mycobacterium tuberculosis} (384 for FtsL and 228 for FtsB), due to N- and/or C-terminal extensions consisting of mostly charged and polar amino acids or proline-rich sequences. The major part of FtsL or FtsB(DivIC) is extracellular and contains a region proximal to the transmembrane segment, predicted to form a coiled-coil of about five heptads. Coiled-coil helices associate longitudinally to mediate protein association. It is possible that the coiled-coil helices are continuations of the transmembrane helices, although a proline (known to break helices) is present in the coiled-coil helices are continuations of the transmembrane segment, the POTRA domain. The POTRA domain of FtsL and DivIC, to give rise to KL and EC fusion proteins, respectively. The constrained dimer (KL/EC) was shown to interact with the extracellular part of DivIB (DivIB\text{ext}), yielding a soluble complex amenable to structural studies (11).

The overall shape of the complex and its constituents was probed using small-angle x-ray scattering (SAXS)\(^2\) and small-angle neutron scattering (SANS). NMR was used to investigate the interface between the proteins by chemical shift mapping. The interaction was further investigated using surface plasmon resonance with truncated forms of the proteins. The complex of DivIB, FtsL, and DivIC is formed by the interaction of one face of the \( \beta \)-domain of DivIB with the C-terminal regions of FtsL and DivIC, at the tip of an elongated rod formed by the coiled-coil segments. The \( \alpha \)-domain of DivIB and the coiled-coil regions of FtsL and DivIC remain free to interact with other proteins of the division apparatus.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—Proteins were produced in \textit{E. coli} from expression plasmids inducible with isopropyl \( \beta \)-d-1-thio-galactopyranoside. The sequence encoding DivIB\text{ext} preceded by the sequence for a tobacco-etched virus protease cleavage site (TEV) was introduced into pGEX-4T1 (GE Healthcare) to produce the fusion protein glutathione S-transferase (GST)-TEV-DivIB\text{ext} (11). The sequence encoding the \( \beta \gamma \)-fragment (residues 224 to 396) was similarly introduced into pGEX-4T1 to produce GST-TEV-\( \beta \gamma \). The sequence encoding the \( \beta \)-domain (residues 224 to 361) following a TEV site was introduced into pLX06 (Protein Engineering, Meriden, Conn.) to produce the fusion protein GST-TEV-\( \beta \).

To produce the KL protein (residues 44 to 105 of FtsL fused following the k5 peptide) with an N-terminal poly-His tag, and a tetryptophan to generate absorption at 280 nm, the codon TGG was introduced by site-directed mutagenesis into the pETduet-based (Novagen) plasmid described previously (11). To produce KL* (as KL but ending at residue 82 of FtsL), the stop codon TAG was introduced by site-directed mutagenesis. To produce EC (residues 55 to 122 of DivIC fused following the e5 peptide) with an N-terminal Strep tag (IBA), the sequence encoding Strep-EC was amplified from the plasmid described previously encoding a poly-His-tagged EC (11) and introduced in pET30 (Novagen). The sequence encoding a poly-His tag followed by a TEV site and EC was similarly introduced into pET30 (Novagen), whereas the sequence encoding a poly-His tag followed by a TEV site and EC* (as EC but ending at residue 93) was introduced into pLIM09 (RoBioMol). A plasmid was also constructed for coexpression of KL and EC by subcloning the sequence encoding the Strep-tagged EC into the pETduet-based plasmid encoding the poly-His-tagged Trp-containing KL.

**Protein Production and Purification**—Unlabeled proteins were expressed in \textit{E. coli} BL21(DE3) RIL in flasks or in a fermentor (Minifors, Infors) in Luria broth or Terrific broth and the appropriate antibiotic at 37 °C following the addition of 0.5 mM isopropyl \( \beta \)-d-1-thio-galactopyranoside. For the production

\(^2\) The abbreviations used are: SAXS, small-angle x-ray scattering; SANS, small-angle neutron scattering; POTRA domain, polypeptide transport-associated domain; TEV, tobacco-etched virus; GST, glutathione S-transferase; Ni-NTA, nickel-nitritoltriacetic acid; PDB, Protein data bank; HSQC, heteronuclear single quantum coherence.
tion of 15N-labeled DivIBextr cells were grown in a fermentor at 28 °C in minimal medium (KH2PO4, 3 g/liter; Na2HPO4, 6 g/liter; NaCl, 0.5 g/liter; MgSO4, 1 mm; FeCl3, 50 μM; CaCl2, 20 μM; MnCl2, 10 μM; ZnSO4, 10 μM; CoCl2, 2 μM; CuCl2, 2 μM; NiCl2, 2 μM; Na2MoO4, 2 μM; H3BO3, 2 μM; biotin, 1 μM; niacinamide, 5 μM; pyridoxine, 5 μM; thiamine, 5 μM; folic acid, 1 μM; riboflavin 1 μM; glucose, 4 g/liter) with 15NH4Cl, 1 g/liter, as nitrogen source. Induction was with 1 mm isopropyl β-D-1-thiogalactopyranoside for 3 h at 30 °C. For the production of the 15N/13C-labeled β-domain, cells were grown in minimal medium as above in D2O with 15NH4Cl, 1 g/liter, as nitrogen source. For the production of 15N/2H-labeled β-domain, cells were grown as above in D2O with 15NH4Cl, 1 g/liter, and [13C]glucose, 4 g/liter, as nitrogen and carbon sources.

DivIBextr, β, and βγ were purified in a first step by glutathione affinity chromatography. Cell lysates in 20 mM Tris, pH 8, 500 mM NaCl, containing the GST-TEV-DivIBextr, GST-TEV-β, or GST-TEV-βγ fusion proteins were loaded onto glutathione-Sepharose resin (GE Healthcare). The fusion proteins were cleaved on the resin by incubation at 37 °C with the poly-His-tagged TEV protease and recovered by washing with 20 mM Tris, pH 8, 150 mM NaCl. The TEV protease was then removed by binding on Ni-NTA resin (Qiagen). This procedure was sufficient for the 15N-DivIBextr protein used for recording [15N-1H]HSQC spectra. 15N-2H- and 15N-2H-13C-labeled β-domains were further purified by size exclusion chromatography on a Superdex 75 column (16 × 600 mm) equilibrated with 20 mM Tris, pH 8, 150 or 20 mM sodium phosphate, pH 6, 150 mM NaCl.

Further purification steps were also applied before scattering experiments. DivIBextr, β, and βγ proteins from the affinity purification were dialyzed against 20 mM Tris, pH 8, to lower the NaCl concentration to 15 mM, prior to loading onto a Resource Q resin (GE Healthcare) and elution with a NaCl gradient from 15 to 500 mM in 10 column volumes. Fractions of interest were pooled and concentrated with Amicon Ultra devices (Millipore) prior to size exclusion chromatography on a Superdex 75 column (10 × 300 mm) (GE Healthcare). Homogenous fractions (as judged by SDS- and nondenaturing polyacrylamide gel electrophoresis) were pooled, dialyzed against 20 mM Tris, pH 8, 100 mM NaCl, and finally concentrated with Amicon Ultra devices.

The protein EC with a Strep-tag was purified from the cell lysate in 20 mM Tris, pH 8, 150 mM NaCl by affinity chromatography on a streptactin resin (IBA). The protein was eluted with 2.5 mM desthiobiotine in the same buffer.

The proteins EC, KL, EC*, and KL* with poly-His tags were purified from cell lysates in 20 mM Tris, pH 8, 500 mM NaCl, 20 mM imidazole, by affinity chromatography on Ni-NTA resin (Qiagen). Proteins were eluted with an imidazole gradient of 20 to 500 mM in 10 column volumes.

The (KL/EC) complex, where EC has a Strep tag and KL a poly-His tag, was prepared from a lysate of cells co-expressing both proteins. The complex was isolated by two successive steps of affinity chromatography in 20 mM Tris, pH 8, 150 mM NaCl, first on Ni-NTA resin eluted with an imidazole gradient from 20 to 500 mM, then on streptactin eluted with 2.5 mM desthiobiotine. Following concentration with an Amicon Ultra device, the complex was further purified by size exclusion chromatography on a Superdex S200 column (16 × 600 mm) equilibrated with 20 mM Tris, pH 8, 150 mM NaCl. For the transverse relation optimized spectroscopy NMR experiment, the buffer was exchanged by desalting on a PD10 column (GE Healthcare) against 100 mM ammonium acetate, pH 7, prior to lyophilization.

Determination of Protein Concentrations—Protein concentrations were determined by the absorbance at 280 nm using the following theoretical extinction coefficient: 5500 M−1 cm−1 for poly-His-tagged KL and KL*, 17,420 M−1 cm−1 for Strepc, EC, 13,410 M−1 cm−1 for poly-His-tagged EC, 4,470 M−1 cm−1 for poly-His-tagged EC*, 26,360 M−1 cm−1 for DivIBextr, 19,858 M−1 cm−1 for βγ, and 11,920 M−1 cm−1 for β. The absorbance spectra were identical without and with 8 M urea indicating that the calculated extinction coefficient could be used without correction. For the KL/EC complex (purified after coexpression of poly-His-tagged KL and Strepc) an amino acid analysis gave an experimental extinction coefficient of 24,740 M−1 cm−1, which is 8% larger than the calculated extinction coefficient (22,920 M−1 cm−1); we used the theoretical coefficient.

NMR Experiments—Exploratory heteronuclear NMR data were collected at 27 °C in a 20 mM Tris buffer (90% (v/v) H2O, 10% (v/v) D2O) at pH 8 with 150 mM NaCl, on samples of DivIBextr at 140 μM, DivIBextr(KL/EC) at 110 μM, and β at 270 μM. Spectra were recorded on a 800 MHz Varian spectrometer equipped with a cold probe. For backbone assignment, a sample of the uniformly 13C/15N-labeled β-domain at 0.8 μM was prepared and data were collected at 27 °C in a 20 mM sodium phosphate buffer (90% (v/v) H2O, 10% (v/v) D2O) at pH 6 with 150 mM NaCl. A set of high field triple resonance experiments (HNCO, HNCA, HNCAcβ, and CβCaCONH) where acquired and assignment was done using the CO, Caα, and Cβ resonances. First assignment was obtained by an automatic approach using MARS (30) and was then refined manually.

The binding of (KL/EC) to the β-domain was studied by transverse relation optimized spectroscopy-type 1H-15N correlation experiments. NMR data were collected at 25 °C with 32 transients and 1024 (1H) × 64 (15N) complex points. A reference spectrum was taken on a 0.4 μM uniformly 13C/15N-labeled deuterated β-domain sample and lyophilized (KL/EC) was added to a concentration of 0.5 mM.

SAXS and SANS Experiments and Data Processing—The scattered intensities I(Q) were analyzed as a function of the wave vector transfer Q, with Equation 1,

\[ Q = (4\pi/\lambda)\sin \theta \]  

where 2θ is the scattering angle and λ the x-ray or neutron wavelength.

The SAXS measurements were performed on the ID02 beamline (ID2 high brilliance beamline) at the European Syn-
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chrotron Radiation Facility (Grenoble, France) at a sample to
detector distance of 2 m to cover the Q-range from 0.008 to 0.28 Å⁻¹. The x-ray wavelength was 1 Å and data were collected by
a high sensitivity CCD (FReLoN) detector placed in an evacu-
ated flight tube. Solutions were loaded in a flow-through quartz
capillary cell (diameter, 2 mm; wall thickness, 10 µm) at 15 °C.
The radiation damage was checked with 10 successive exposure
times of 0.1 s each on a single part of the sample. Longer expo-
sure time led to damage. Therefore, we acquired and averaged
10 curves of 0.1-s exposure time on different parts of the sample
that was pushed through the capillary cell. The two-dimen-
sional diffraction patterns were normalized to an absolute scale
density in cm⁻³, and azimuthally averaged to obtain the intensity profiles \( I(Q) \), and the solvent background was subtracted using Fit2D.

SANS was carried out on the large dynamic range small-
angle diffractometer D22 at the Institut Laue-Langevin
(Grenoble, France). 200-µL Samples in H₂O buffers were mea-
ured in Hellma® QS 1-mm quartz cells at 4 °C. (Data sets from samples in D₂O could not be used due to aggregation prob-
lems.) The sample to detector distances were 2 and 8 m with an
incident neutron wavelength of 6 Å to cover a Q-range from
0.025 to 0.30 Å⁻¹. The sample and buffer raw two-dimensional
intensities (after masking inappropriate data points) were nor-
malized to the incoming neutron flux and corrected for detec-
tor efficiency (H₂O reference), electronic background (B₁C standard), and sample holder scattering (empty quartz cuvette),
using RNILS (34). Corrected two-dimensional sample and buffer
intensities were summed up azimuthally into one-di-
dimensional \( I(Q) \) intensities using SPOLLY (34). Buffer intensi-
ties were then subtracted using PRIMUS (35).

Determination of Molecular Weights—Molecular masses were determined by SAXS relatively to hen egg white lysozyme
using Equation 2,

\[
M_r = \frac{I(0)}{f_2 C} \frac{1}{T} \tag{Eq. 2}
\]

where \( I(0) \) is the forward scattering and \( C \) is the protein con-
centration in g/liter. The factor \( f_2 = (83 \pm 1) \times 10^{-4} \) mol L⁻² g⁻²
was determined using hen egg white lysozyme at a concentra-
tion of 10.0 g/liter (36).

Molecular weights were determined on an absolute scale by
SANS using Equation 3 (37),

\[
M_r = \frac{1 - T}{4\pi N_A} \frac{I(0)}{I_{inc}(0)} \frac{10^4}{CN_A} \left[ \sum b_i - \rho_p V/M_r \right]^{-2} \tag{Eq. 3}
\]

where \( I(0) \) is the coherent macroscopic forward scattering
(extracted by the Guinier approximation, see Equation 4),
\( I_{inc}(0) \) is the incoherent forward scattering from H₂O, \( T_r \) and \( T \)
are the transmissions of the sample and H₂O, respectively. \( C \) is
the protein concentration in g/liter, \( t \) is the sample thickness in
cm, \( f \) is a correction factor for the anisotropicity of the solvent
scattering as a function of neutron wavelength. \( b_i \) are the scat-
ttering lengths of the protein atoms in cm, \( \rho_p \) is the solvent scat-
ttering density in cm⁻², \( V \) is the protein excluded volume, and
\( N_A \) is the Avogadro constant.

Determination of the Radii of Gyration—We extracted the
radii of gyration \( R_g \) of all samples by a Guinier analysis using
Equation 4 (38),

\[
\ln[I(Q)] = \ln[I(0)] - \frac{1}{3} R_g^2 Q^2 \tag{Eq. 4}
\]

where \( I(0) \) is the intensity scattered in the forward direction.
The \( I(0) \) and \( R_g \) values were extracted by Guinier approxima-
tion using the program PRIMUS (35). The validity of the
approximation was checked \textit{a posteriori} in each case and was
always fulfilled (\( R_g^2 Q_{(max)} < 1 \)).

The cross-sectional radius of gyration \( R_C \) was determined according to Equation 5,

\[
l_c(Q) = l_c(0)\exp\left(-\frac{1}{2}Q^2R_C^2\right) \tag{Eq. 5}
\]

with \( l_c(Q) = I(Q)Q/\pi L \), with a graphical fit using the program
PRIMUS (35). The good agreement between the radii of gyration
and the maximum extension of the particle \( (L = D_{max}) \) was
checked using the relationship in Equation 6.

\[
\frac{L^2}{12} = R_g^2 - R_C^2 \tag{Eq. 6}
\]

Distance Distribution Functions and \textit{ab Initio} Modeling—The
distance distribution functions \( P(r) \) were determined by the
program Gnom (39) from SAXS and SANS curves. The boundary
conditions \( P(0) = P(D_{max}) = 0 \) were imposed in each case, \( D_{max} \)
being the maximum extension of the particles. Low-resolution
envelopes were calculated from the \( P(r) \) functions with DAM-
MIN (40).

Rigid Body Modeling—The experimental SANS data from
the complex \( \beta \gamma (KL/EC) \) were modeled using the \( \beta \gamma \textit{ab initio} \)
model and the coiled-coil structure of cortexin I (Protein Data
Bank entry 1D7M) as rigid bodies using the program MASSHA
with default parameters (41). In addition, several arrangements
of both rigid bodies were generated manually using MOLMOL
(42) and scored against the SANS curve using CRYSON (43).

Calculation of Scattering Intensities from Known Structures—
Theoretical scattering curves \( I(Q) \) of the \textit{E. coli} and \textit{G. stearo-
thermophilus} \( \beta \)-domains were calculated from PDB entries
2VH1 and 1YR1 using the program CRYSOL (44) with
default parameters.

Surface Plasmon Resonance Measurements—Measurements
were performed with a Biacore™ 3000 instrument. All samples
were dialyzed against 10 mm Hepes, pH 7.5, 150 mm NaCl, 50
mm EDTA, and 0.005% P20. The dialysis buffer was used as
running solution throughout. KL/EC, KL*/EC, and KL/EC*
mixtures (with all proteins carrying an N-terminal poly-His tag)
were attached to a NTA sensor chip activated with 10 µL of 500
µM NiCl₂. Experiments were at room temperature with a 20-µL/
min flow rate. The (KL/EC), (KL*/EC), and (KL/EC*) dimers
(ligands) were injected at the concentration of 100 nm. The
DivIBext, \( \beta \) and \( \beta \) proteins (analytes) were injected at con-
centrations ranging from 5 µm to 1.5 mM. For each experiment,
interaction curves recorded for the running buffer on the
ligand, and for the analyte on the naked sensor chip, were
subtracted. Sensorgrams were analyzed with the program
BLAevaluation 3.0.
RESULTS

Sequence Comparisons—The coiled-coil regions of FtsL and DivIC(FtsB) are usually followed by 25 to 30 amino acids that are C-terminal, except in species with long additional unfolded extensions. There is little sequence conservation between species, and it is sometimes difficult to identify the genes encoding FtsL or DivIC(FtsB) using sequence comparison methods. Instead, it is best to look for genes encoding proteins with a coil region, in the proper chromosomal environment. The ftsL and ftsB完整的proteins are usually following the coiled-coil region, and lack the conserved residues mentioned above. Remarkably, the coiled-coil regions were predicted using whole alignments with the program TMAP. The aligments are presented in supplemental Figs. S1 and S2.

Despite the absence of overall sequence conservation of FtsL, three hydrophobic amino acids (a Val/Leu/Ile, an Ala, and a Leu/Met) were conserved in the region following the predicted coiled-coil (supplemental Fig. S1). In FtsB(DivIC), a basic (Arg) and an acidic (Glu) residue were conserved in the region following the predicted coiled-coil region, in the proper chromosomal environment. The ftsL gene is usually following mraW, whereas divIC(ftsB) is usually close to that of enolase.

A set of FtsL and DivIC(FtsB) sequences were aligned using the program CLUSTALW. The transmembrane region was predicted using whole alignments with the program TMAP. The coiled-coil regions were predicted using PAIRCOIL2. The alignments are presented in supplemental Figs. S1 and S2.

It is noteworthy that the extracellular region of both FtsL and FtsB are shorter in Campylobacter, with very little sequence following the predicted coiled-coil region, and lack the conserved residues mentioned above. Remarkably, Campylobacter also appear devoid of FtsQ, suggesting that the C-terminal regions of FtsL and FtsB(DivIC) containing the conserved residues might mediate the interaction with (FtsQ)DivIB.

Limited Proteolysis—To probe the domain organization of DivIB from S. pneumoniae, we submitted DivIBext (corresponding to residues 150 to 396) to trypsin digestion. A time course monitored by Coomassie-stained SDS-polyacrylamide gel electrophoresis is shown in Fig. 1. DivIBext (28,177 Da) migrated as a 28-kDa protein. After 50 min of digestion with trypsin, a major stable fragment migrated as a 15-kDa protein. The mass was determined by electrospray mass spectrometry to be 15,842 ± 1 Da. N-terminal sequencing yielded the sequence VKEYDIVA. Combining the mass and sequence data indicated that the fragment spans residues 220 to 361 of pneumococcal DivIB (15,843 Da). This fragment overlaps the β-domain defined by the structure of FtsQ from E. coli, and is somewhat longer on the C-terminal side than the β-domain defined by partial proteolysis of DivIB from G. stearothermophilus. The trypsin-sensitive regions 150 to 219 and 362 to 396 correspond to regions α and γ, respectively.

For further studies, two truncated forms of pneumococcal DivIB that we termed β and βγ, corresponding to residues 224 to 361 and 224 to 396 C terminus, respectively, were purified. We decided to produce proteins with a truncated N terminus with respect to our proteolysis results to exclude the fully conserved Glu-222, which is absent from the β-domain from G. stearothermophilus, and appears to be part of the linker between the α- and β-domains from E. coli and Y. enterocolitica. Both β and βγ contain an additional N-terminal glycine resulting from cleavage of the fusion with the GST using the TEV protease.

NMR Investigations of the Extracellular Part of DivIB and Its Interaction with a Soluble Artificially Constrained Dimer of FtsL and DivIC—To gather information on the interaction between DivIB and the (FtsL/DivIC) dimer, we undertook NMR studies of the soluble model systems consisting of the complete extracellular DivIBext or the β-domain, with or without the presence of the soluble dimer (KL/EC). The [15N-1H]HSQC spectrum of 15N-labeled DivIBext showed about 100 well dispersed isolated peaks, as well as an area of unresolved peaks in the vicinity of 120 and 8 ppm, corresponding to the 15N and 1H dimensions, respectively (not shown). [15N-1H]HSQC spectra show resonance peaks corresponding to every proton attached to 15N atoms, i.e. the amide protons of the backbone chain and the few amine protons from side chains. Amide protons from unstructured polypeptides present a low dispersion of the resonances centered for the proton around 8 ppm. Amide protons in folded structures are each in particular environments that modify their resonance frequencies (chemical shift) resulting in the isolation and spreading of their peaks. The spectrum therefore indicated that the 247-residue long DivIBext contains a folded domain of about 100 amino acids, and about 150 unstructured residues.

The [15N-1H]HSQC spectrum of the β-domain showed about 100 well dispersed peaks superimposable to those of DivIBext (not shown), without an accumulation of resonances in the 120- or 8-ppm regions. The comparison of the spectra of DivIBext and β showed that the trypsin-sensitive α- and γ-regions were largely unstructured.

When an excess of unlabeled (KL/EC) was added to 15N- DivIBext, at a concentration where most of the DivIBext should be interacting with the dimer, the [15N-1H]HSQC spectrum showed the disappearance of nearly all the dispersed peaks and the persistence of the peaks due to unfolded parts of the protein (not shown). We interpreted these observations as indicating that the (KL/EC) dimer interacts with the β-domain of DivIB, without important contact with the α- or γ-regions. When the same experiment was performed with only the 15N-β-domain,
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all the peaks disappeared. The disappearance of the peaks from the β-domain upon association with (KL/EC) could be explained by a greater transverse relaxation resulting from a change in the hydrodynamic behavior of the protein. The β/(KL/EC) complex likely assumes a non-compact shape that drastically reduces its rotational diffusion. If the complex were compact, the 2-fold difference in size between the free β-domain and β/(KL/EC) would not reduce the rotational diffusion to the point of extinguishing the signal. The persistence of the signal of the α- and γ-regions observed on the complete \[^{15}N\text{-DivIB}_{\text{ext}}\] in the presence of (KL/EC) indicates that these do not participate in the interaction, and that they retain their flexibility.

The disappearance of the signal upon formation of the ternary complex precluded further use of the \[^{15}N\]-labeled samples. To minimize the translational relaxation, and thus compensate the loss of signal due to the loss of rotational diffusion upon formation of the ternary complex, we prepared a \(^{15}N/2H\) doubly labeled sample of β. The overall deuterium reduces the translational relaxation, whereas atoms that can be exchanged with \(^1H\) protons from the solvent, such as amide protons, remain visible in \[^{15}N-1H\]HSQC experiments.

Indeed, the \[^{15}N-1H\]HSQC spectrum of the \[^{15}N/2H\]-β-domain was identical to that of the \[^{15}N\]-labeled sample. In contrast to what had been observed with the \(^{15}N\)-labeled β-domain, it was still possible to record a good quality spectrum following addition of an excess of KL/EC to the \[^{15}N/2H\]-β-domain (Fig. 2A). The spectrum of the free β-domain exhibited 126 resolved peaks at 27 °C. The spectrum of β in the presence of (KL/EC) revealed a modification of the chemical shift for 27 peaks. The modification of the chemical shift of some protons of the β-domain indicates that their local environment is changed in the complex with (KL/EC). To identify the position of these protons, it was necessary to assign the peaks to specific protons of the protein. For this purpose, we produced a doubly labeled \(^{13}C/^{15}N\)-sample of the β-domain. Assignment of the \(^1H\) resonance peaks was achieved by analyzing HSQC, HNCO, HNCA, HNCA, CBCACONH spectra. Of the 126 peaks of the \[^{15}N-1H\]HSQC spectrum, 97 could be assigned, including 27 that experienced modification of their chemical shift upon binding of (KL/EC). The attribution is nearly complete for the first three quarters of the β-domain (residues 224 to 323) but could not be achieved for residues 324 to 352 (Fig. 2B), probably due to the dynamics of conformational exchanges of the region. Secondary structure propensities obtained from the \(^{13}C_\alpha\) and \(^{13}C_\beta\) chemical shifts of the pneumococcal protein, compared with the secondary structures of the E. coli and G. stearothermophilus proteins, are given in supplemental Fig. S3.

Small-angle X-ray Scattering Investigation of the Extracellular Part of DivIB and (KL/EC)—To obtain low-resolution structural information on the extracellular part of DivIB in solution, a series of small-angle x-ray scattering measurements were performed on various truncated forms of the protein.

Small-angle scattering curves provide information on the mass and shape of the scattering particle. The mass is proportional to the intensity scattered in the forward direction \((I(0))\) provided that the sample is dilute and that there is a single scattering species, and can be extracted from SANS and SANS data using Equations 2 and 3, respectively (see “Experimental Procedures”). The shape of the scattering curve \(I(Q)\) is related to the shape of the particle and yields the radius of gyration, \(R_g\) at the smallest scattering angles (Guinier approximation, see Equation 4 under “Experimental Procedures”). The distribution of distances between scattering points of the particle (termed distance distribution function \(P(r)\)) can be extracted from the whole scattering curve. Combined, the radius of gyration, the profile of the distance distribution function, and the molecular mass hint at the shape of the particle (e.g. elongated versus compact). Theoretical distance distribution functions and scattering curves can be unambiguously calculated for any particle of known atomic structure. The reverse is not true, but three-dimensional models of a particle can be constructed by iterative numerical simulation.

The complete extracellular part of DivIB\(_{\text{ext}}\) produced scattering curves with very high forward scattering \((I(0))\) (not shown), indicating that samples were not monodisperse but contained oligomers or aggregates. Masses determined from the \(I(0)\) values increased with the protein concentration. The presence of aggregates of DivIB\(_{\text{ext}}\) were also observed by analytical ultracentrifugation (not shown). The formation of aggregates was not unexpected given that DivIB\(_{\text{ext}}\) is partly unfolded.
but precluded the use of SAXS or SANS for further investigation.

The shape of the SAXS curves obtained for the β and βγ proteins showed no variation with the concentration (between 117 and 685 μM for β, between 33 and 313 μM for βγ). The apparent masses determined from the I(0) values were consistent with the theoretical masses. The measured mass for β was 16 ± 2 kDa for an expected mass, M_{theor} = 15 kDa, that of βγ was 25 ± 3 kDa for an expected mass, M_{theor} = 20 kDa. The shape of the scattering curves and the good agreement between measured and calculated masses showed that the samples were largely monodisperse, as observed by analytical ultracentrifugation (not shown).

For comparison, theoretical scattering curves were calculated for the β-domain of DivIB from *G. stearothermophilus* (residues 115 to 233) and FtsQ from *E. coli* (residues 126 to 260) using the program CRYSOl (44). The normalized experimental and theoretical curves are shown in Fig. 3A. The experimental SAXS curve of the β-domain from *S. pneumoniae* is very similar to that calculated for the *E. coli* orthologue, indicating that both protein domains have the same overall shape. However, the βγ-protein has the same shape as the β-domain with a longitudinal extension.

*Ab initio* models of the β- and βγ-proteins were calculated from the distance distribution functions. Five independent models were produced and were consistent (Figs. 3C and supplemental S4). The resolution of the β models is coarser than that of βγ, as the data were noisier at large Q-values. One representative model of each pneumococcal truncated form (β and βγ) is shown in Fig. 3C, alongside structures of the β-domain from *G. stearothermophilus* and *E. coli*. The β-domain of pneumococcal DivIB appears to be elongated and slightly curved, or “bean”-shaped. Models of the βγ protein fragment comprise a bean-shaped domain and an additional smaller globular domain attached by a short stalk at one extremity of the bean, which likely constitutes the γ-tail. The γ-region is unstructured as judged by its sensitivity to trypsin and the NMR data. The smaller globular domain of the βγ model could represent conformational sampling by the unfolded γ-tail.

As expected from direct comparison of the SAXS and theoretical scattering curves, the pneumococcal β-domain has an overall shape similar to that of the *E. coli* β-domain. The crystal
structure of the E. coli β-domain can be placed easily within the model envelope of the bean-shaped domain of the βγ-fragment (Fig. 3D).

Relative molecular mass measurements by SAXS of the (KL/EC) artificially constrained dimer at various concentrations indicated that the dimer was self-associating to form a tetramer (KL/EC)\(_2\) with a dissociation constant comprised between 5 and 50 μM (not shown). We did not pursue structural characterization of the (KL/EC)\(_2\) species as it is likely an artifact resulting from higher order association of the k5/e5 coiled-coil, which is known to form (k5/e5)\(_2\) tetramers at high concentrations (29). Note that hydrodynamic techniques such as size exclusion chromatography, dynamic light scattering, or analytical ultracentrifugation failed to reveal the formation of the tetramer (KL/EC)\(_2\), presumably because the (KL/EC) and (KL/EC)\(_2\) particles have comparable hydrodynamical properties despite having a 2-fold mass difference, due to the elongated, highly anisotropic nature of the coiled-coils. Small-angle Neutron and X-ray Scattering Investigations of the Ternary Complex βγ/KL/EC—At high concentrations of βγ/KL/EC (>10 μM), necessary to generate high quality scattering data, we could have expected the presence of species such as βγ(KL/EC)\(_2\) or βγ\(_2\)(KL/EC)\(_2\), of unknown biological significance. The formation of (KL/EC)\(_2\) being unknown to us at the time, we nevertheless acquired SANS and SAXS curves of KL/EC mixtures, βγ alone, and βγ/KL/EC.

Aggregation in 42 and 100% D\(_2\)O prevented using the differential deuteration of subunits in various H\(_2\)O/D\(_2\)O solutions to identify the individual components within the complex, as initially planned. A SANS experiment in water is presented here and data are summarized in Table 1. The mass of the scattering species in the KL/EC sample (55 ± 8 kDa) was consistent with that of the tetramer (KL/EC)\(_2\) (\(M_{\text{theor}} = 50 \text{ kDa}\)), as expected from the SAXS results for a concentration of 270 μM. The measured mass of the βγ-fragment (22 ± 5 kDa) was also consistent with that of a monomer (\(M_{\text{theor}} = 20 \text{ kDa}\)). The mean mass of the scattering species in the sample containing stoichiometric amounts of KL/EC and βγ-fragment (46 ± 6 kDa) did not correspond to that of a complex (βγ)\(_2\)(KL/EC)\(_2\) (\(M_{\text{theor}} = 90 \text{ kDa}\)), nor to a noninteracting mixture of βγ and (KL/EC)\(_2\) (mean \(M_{\text{theor}} = 35 \text{ kDa}\)). Instead, the measured mass was consistent with that of the βγ(KL/EC) complex (\(M_{\text{theor}} = 45 \text{ kDa}\)), or possibly with a mixture of βγ(KL/EC)\(_2\) and free βγ (mean \(M_{\text{theor}} = 45 \text{ kDa}\)). Analytical ultracentrifugation of the sample showed the presence of a single species (not shown), indicating that the acquired SANS curve was likely that of the ternary βγ(KL/EC) complex.

The masses measured by SANS suggested that the association with the βγ-protein could drive the dissociation of the tetramer (KL/EC)\(_2\) into the (KL/EC) dimer. The reaction could be described by the two competing equilibria, βγ + (KL/EC) ⇌ βγ(KL/EC) with the constant \(K_{D1}\), and (KL/EC)\(_2\) ⇌ 2(KL/EC) with the constant \(K_{D2}\). As we had values of the equilibrium constants \(K_{D1} = 0.22 \mu\text{M}\) for dissociation of the βγ(KL/EC) complex determined by surface plasmon resonance (see below), and \(K_{D2}\) comprised between 5 and 50 μM for dissociation of (KL/EC)\(_2\), estimated from SAXS mass measurements, we ran numerical simulations of the composition of the mixture at equimolar concentrations of βγ and (KL/EC). The simulations showed that for concentrations greater than 100 μM, the sample would contain mostly the βγ(KL/EC) complex, with negligible amounts of free species and (KL/EC)\(_2\) tetramer (supplemental Fig. S5).

We therefore decided to analyze the SANS curve from the sample containing equimolar amounts (270 μM) of βγ and KL/EC as represented by the βγ(KL/EC) complex. The scattering curve I(\(q\)) has a rather featureless profile (Fig. 4A) typical of elongated particles. The radius of gyration \(R_g\) was found to be 47 ± 3 Å. We have also determined the cross-sectional radius of gyration \(R_c\) to be 9.7 ± 0.6 Å (supplemental Fig. S6). The profile of the distance distribution function \(P(r)\) is similar to those produced by rod-like structures (45), with a steep increase at short distances and a slow decay at larger distances (Fig. 4B). The optimal \(D_{\text{max}}\) was found to be 160 Å, which is consistent with the \(R_g\) and \(R_c\) values (see Equation 6 under "Experimental Procedures"). Five independent low resolution models of the βγ(KL/EC) complex were calculated ab initio from the SANS curve (Figs. 4C and supplemental S7). All the models are very elongated and straight with a small cross-section diameter. A common feature found in all models is an excess of mass near one end of the elongated structure. The long dimension of the excess density is about 60 Å. It is tempting to interpret this bulge as the βγ-protein sitting near the tip of the (KL/EC) coiled-coil dimer.

The SAXS curve of a sample containing equimolar quantities of βγ and KL/EC (144 μM) was very similar to the SANS curve (Fig. 4A). Because of the short exposure time due to radiation damage, quality of the SAXS data were not significantly better than that of the SANS experiments.

We also carried out rigid body modeling of the complex. Rigid body modeling seeks to minimize iteratively the difference between the experimental curve and the theoretically calculated curve of the model by translating and rotating the two partners as rigid bodies in a grid search. As rigid bodies, we used the low-resolution model of the βγ-fragment based on the SAXS data, and a high-resolution model of the dimerization domain of the cortexillin I from Dictyostelium discoideum as a proxy for the (KL/EC) coiled-coil dimer. The parallel homodimer coiled-coil of cortexillin comprises 200 amino acids (14 heptads) that form a long rod, which we used as a model of (KL/EC) that totals 216 amino acids and is predicted to contain at least 10 heptads plus N- and C-terminal extensions (the purification tags and the regions containing the few conserved amino acids, respectively). Fig. 4D shows five different models that fit the experimental SANS scattering curve equally well. In all models, the βγ-protein is positioned near the end of the elongated partner, their long axis being roughly aligned.

| TABLE 1 | Forward scattering values and associated apparent masses from SANS measurements of KL/EC, βγ and KL/EC/βγ samples |
|----------|----------------------------------------------------------------------------------------------------------|
| Values and mass | (βγ) (μM) | (KL/EC) (μM) | I(0) | \(M_{\text{app}}\) (kDa) |
|----------|-------------|----------------|------|---------------------|
| 0         | 270         | 270            | 0.201 ± 0.010 | 55 ± 8 |
| 270       | 270         | 0.305 ± 0.010  | 46 ± 6 | 22 ± 5 |
| 340       | 0           | 0.085 ± 0.009  | 22 ± 5 |
The rigid body modeling did not allow discrimination between the two alternative orientations (head up or head down) of the βγ-fragment relative to the (KL/EC) rod.

Manual sampling of alternative positions of the βγ-protein (e.g. at the extreme tip or in the middle of the rod) and orientations (e.g. perpendicular to the rod) yielded theoretical curves with worse fit to the data (supplemental Fig. S8).

**Interaction between the Extracellular Part of DivIB and KL/EC Investigated by Surface Plasmon Resonance**—To evaluate the affinity of DivIB for the dimer (FtsL/DivIC) we used surface plasmon resonance (Biacore technology), where one partner (the ligand) is attached to a sensor chip, whereas the other interacting partner (the analyte) is presented over the sensor chip in a flowing solution. The surface plasmon resonance being sensitive to the mass of the particles attached to the surface of the sensor, the association kinetics can then be measured. The dissociation kinetics can be measured afterward by flowing a solution without analyte.

For the surface plasmon resonance experiments, the (KL/EC) complex was attached to the sensor chip by the N termini to present the FtsL/DivIC moieties to the solution. Attachment was achieved on Ni²⁺-NTA chips with both KL and EC proteins harboring an N-terminal poly-His tag. The KL/EC mixture was injected onto the sensor chip at a concentration where the (KL/EC) complex is a dimer, and not a tetramer. Association and dissociation curves could be recorded for the three soluble forms: DivIBext, βγ, and βγ. The βγ- and βγ-truncated forms produced comparable interaction curves with (KL/EC) (not shown).

We measured the kinetic parameters in the case of the βγ-protein. Data are shown in Fig. 5. The theoretical curves describing the interaction according to a simple Langmuir model of association, βγ + (KL/EC) ⇄ βγ(KL/EC), were globally fit to the experimental curves.
curves of the
injected at concentrations of 0.08, 0.15, 0.30, 0.55, 0.77, 1.00, and 1.46 mM, DivIBext was injected on the sensor chip devoid of (KL/EC).

A similar linear increase was observed when plateau, but continued to increase linearly, indicating aggregation. The (KL/EC) dimer (400 response units) was attached by its poly-His tags to a Ni-NTA sensor chip. The βγ-protein was injected at concentrations of 0.08, 0.15, 0.30, 0.55, 0.77, 1.00, and 1.46 mM, corresponding to the curves from lowest to highest. The association and dissociation rate constants were extracted by globally fitting theoretical curves to the data according to a simple Langmuir model of association. Thin lines show the theoretical fitted curves.

The extracted association rate constant was $k_A = 7.3 \times 10^4 \text{M}^{-1} \text{s}^{-1}$, the dissociation rate constant $k_D = 0.016 \text{s}^{-1}$, and the equilibrium dissociation constant was $K_D = 220 \text{nm}$, with $\chi^2 = 2.26$.

In the case of DivIBext, the association curve did not reach a plateau, but continued to increase linearly, indicating aggregation (not shown). A similar linear increase was observed when DivIBext was injected on the sensor chip devoid of (KL/EC). However, the aggregation rates on the naked surface and on the (KL/EC)-coated surface were not identical in most experiments, thus precluding subtraction. In one instance, when the linear increase was identical in the presence and absence of (KL/EC), an equilibrium constant of about 300 nm was determined, which is comparable with the constant measured with the βγ-protein.

The value of the affinity of the βγ-fragment for the (KL/EC) dimer has no direct significance for the physiological interaction between DivIB and (FtsL/DivIC), as the proteins in vivo are attached to the plasma membrane and thus restricted in two dimensions. However, the relatively high dissociation constant explains the practical difficulty in isolating the recombinant complex in vitro, as it can be partially dissociated during washing steps of co-purification procedures, or during the course of a size exclusion chromatography.

To examine the importance of the C-terminal regions of FtsL and DivIC in the interaction with DivIB, we produced truncated variants of KL and EC, termed KL* and EC*, and tested the interaction of the KL/EC* and KL*/EC dimer with the βγ-fragment using the surface plasmon resonance assay. The KL and EC proteins were truncated so as to preserve the predicted coiled-coil region (Fig. 6A).

Fig. 6B shows the surface plasmon resonance interaction curves of the βγ-fragment with immobilized (KL/EC), (KL*/ EC), and (KL/EC*). Care was taken to immobilize comparable amounts of each dimer onto the sensor chip. Strikingly, truncation of either KL or EC completely abolished the interaction with the βγ-protein, demonstrating the importance of the C-terminal region of both FtsL and DivIC.

DISCUSSION
DivIB, FtsL, and DivIC form a ternary complex with an enigmatic function that is central to the process of bacterial division. The present work provides the first structural information on the organization of the complex, suggesting new hypotheses.

The crystal structures of the POTRA and β domains from E. coli and Y. enterocolitica FtsQ have been solved (24). In contrast, our NMR and proteolysis results show that the POTRA domain from S. pneumoniae is not folded in the recombinant soluble form of the protein, as was observed with DivIB from G. stearothermophilus (26). We find it unlikely that the DivIB POTRA domain from the two latter species is truly unfolded under physiological conditions, given the conservation of the POTRA motif (25) and the results from sequence hydrophobic cluster analysis that are typical of folded proteins (11). It is more likely that the DivIB POTRA domain has a marginal conformational stability in some organisms, and that this domain is not stable in conditions that differ from those in vivo. Factors that could stabilize the structure of the POTRA domain in vivo include the pH, which may be different close to the membrane.
than in the bulk water, the ionic strength, the charges at the membrane surface, or the presence of interacting protein partners. Indeed, epitopes required for the recruitment of DivIB(FtsQ) to the division site have been found in the POTRA domain, suggesting interactions with other proteins (23, 24).

The low-resolution structure of the $\beta$-domain of pneumococcal DivIB resembles more that from E. coli FtsQ than that from G. stearothermophilus DivIB. The main reason is that the $\beta$-domain from G. stearothermophilus is shorter as it lacks two C-terminal $\beta$-strands (strands S11 and S12, Fig. 7A). Our proteolysis and NMR data concur with the crystallographic results to conclude that the $\beta$-domain includes S11 and S12. The absence of these $\beta$-strands in the $\beta$-domain from G. stearothermophilus is likely the unfortunate consequence of the accessible loop between S11 to the helix H5 containing a trypsin cleavage site, as proposed previously (24).

The NMR spectra of DivIBext with and without (KL/EC) showed that the $\beta$-domain is involved in the interaction. The signal from the unfolded $\alpha$- and $\gamma$-regions were not significantly affected, implying little or no interaction of these parts with the (KL/EC) dimer. However, as proton resonances of the unfolded segments are not resolved, the disappearance of some peaks from this region of the spectrum would pass unnoticed. We therefore cannot rule out completely that formation of the DivIBext-(KL/EC) complex induces some marginal structure in the $\alpha$- or $\gamma$-regions.

Using a deuterated $\beta$-domain to overcome relaxation problems resulting from the hydrodynamic properties of the (KL/EC) complex, it has been possible to detect the modification of chemical shift of 27 amide protons, which could be identified following attribution of the resonances. Given the good agreement between the low-resolution structure of the $\beta$-domain from pneumococcal DivIB and the crystal structure from E. coli and Y. enterocolitica FtsQ, we have tried to locate the positions affected by interaction with (KL/EC). For that purpose, we constructed an atomic model of the $\beta$-domain from S. pneumoniae based on that from Y. enterocolitica using the program SWISS-MODEL (46). As the sequence identity of 10% between the

![Figure 7A](image)

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β-domains of both organisms is very low, the proposed model should be considered with caution and certainly not as a high-resolution structure. However, the hypothetical model presented in Fig. 7, A and B, is likely sufficient to broadly locate the regions of interaction with FtsL and DivIC. Indeed, the sequence alignment between the S. pneumoniae and Y. enterocolitica proteins resulting from the model building, and the classical alignment between the S. pneumoniae and G. stearothermophilus show good agreement for the position of the secondary structure elements (Fig. 7C), lending some credence to the model.

In the model of the S. pneumoniae β-domain, the positions affected by the interaction with (KL/EC) are defining a large surface on the face of the bean that is formed by the β-sheet, whereas the opposite face formed by the α-helices is largely unaffected (Fig. 7, A and B). Previous mutagenesis studies in vivo have implicated the C-terminal β-strands of the β-domain in the recruitment of (FtsL/FtsB) in E. coli (24), and in the function of pneumococcal DivIB likely linked to the interaction with FtsL (17). Unfortunately, we were not able to attribute unambiguously the amide protons of this part of the sequence to NMR resonances (Fig. 2B). The absence of resonance peaks is likely due to conformational exchanges in this region of the protein. Residues 353 to 361 with attributed resonance are not included in the model due to the uncertainty of the alignment in the region. It is therefore possible that the interface with the (KL/EC) dimer extends to the C-terminal region at the “top” of the bean.

In the absence of the high resolution structure of the pneumococcal β-domain, it is premature to test the importance of residues at the interface with FtsL and DivIC by site-directed mutagenesis. As the interface covers a large part of one face of the β-domain, it is possible that single mutations would not be sufficient to suppress the interaction, although D237N and D237E substitutions in E. coli FtsQ each abolished recruitment of FtsL to the division site (24, 47).

The ab initio model of the βγ(KL/EC) complex based on the SANS data, corroborated by rigid body modeling, indicates that the β-domain is positioned near the tip of the rod constituted by the long (KL/EC) coiled-coil. Previous controls had shown that DivIBext does not interact with the artificial K/E part (11). It was therefore reasonable to assume that the β-domain binds to the C-terminal regions of FtsL and DivIC. This result was confirmed by the complete loss of the interaction measured by surface plasmon resonance when either KL or EC were truncated of their C termini.

When viewing the models presented in Fig. 4, it is to be remembered that a large part of the long rod is constituted by the k5 and e5 coiled-coil moieties that were added to constrain the dimerization of the extracellular regions of FtsL and DivIC (11). The purification tags of the KL and EC proteins are also present and contribute to the length of the complex. However, the five predicted heptads of the extracellular coiled-coil of the FtsL/DivIC dimer should extend about 50 Å from the membrane to present the C-terminal regions that contain the conserved residues. The POTRA or α-domain of E. coli FtsQ is about 30 Å long and there are about eight additional residues between the transmembrane segment and the POTRA domain, so that the lower part of the β-domain could stand about 30 to 60 Å from the membrane, whether the eight additional amino acids are tightly folded or fully extended. However, the sequence of the eight residues proximal to the membrane does not have the polar character to constitute a long solvent-exposed extended linker. If the base of the β-domain does not reach 50 Å from the membrane, the (FtsL/DivIC) rod may need to be tilted to present its extremity to the β-domain. An angle of 37° would allow the end of the FtsL/DivIC coiled-coil to make contact with the base of a β-domain 40 Å from the membrane. Note that tilt angles of this magnitude are not unheard of for transmembrane helices (48). Alternatively, the presence of a kink between the transmembrane segment and the extracellular coiled-coil of FtsL and DivIC cannot be excluded. Indeed, the prediction score for a helical structure, using various programs, falls in the segment of a few residues next to the membrane (Fig. 8).
Several genetic studies have failed to indicate a role of the POTRA domain in the recruitment of FtsL and DivIC(FtsB). Also, our NMR data do not support an interaction between the POTRA domain and the (FtsL/DivIC) dimer. If the C-terminal segments of the dimer constitute the sole region that interacts with the β-domain of DivIC(FtsB), the resulting model leaves the coiled-coil rod free for interacting with other proteins. In this respect, it is striking that the Campylobacterial proteins, which divide without FtsQ, have retained the coiled-coil part of FtsL and FtsB. The absence of the C-terminal regions of FtsL and FtsB in Campylobacterial proteins indicates that the coiled-coil region plays a role per se, and not simply as a way to bring the C-terminal parts together. The coiled-coil rod of the FtsL/DivIC(FtsB) dimer, for example, could interact with the septal penicillin-binding protein FtsI, to regulate the function of this enzyme in the assembly of the cell wall. Indeed, a dimer of FtsL and a C-terminal truncated form of FtsB is still able to recruit FtsL
to the division site (27).

It is paradoxical that the coiled-coil part of the FtsL/DivIC(FtsB) dimer appears to be the essential feature of this complex as it exhibits no sequence conservation. This paradox prompts us to propose another speculative function for the dimer. One of the many processes coordinated during bacterial cell division is the membrane invagination. It is possible that the onset of the invagination, which implies a sharp membrane curvature, might require or benefit from destabilization or modification of the lipid bilayer properties. In this respect, it is noteworthy that oligomerization of bitopic membrane proteins, driven by the formation of coiled-coils, is at the core of eukaryotic and viral membrane fusion events (49). Most interestingly, it was found in biophysical studies that oligomerization of a transmembrane fusogenic peptide, constrained by an artificial extramembranous coiled-coil domain, modifies and destabilizes the membrane (31). An analogous phenomenon could occur during bacterial cell division, where the dimerization of FtsL and DivIC(FtsB) might bring their transmembrane segments in close proximity, thereby inducing local modification of the membrane properties, which could be important for the invagination.

In these hypothetical models of the function of FtsL and DivIC, the capping provided by DivIB may simply stabilize the coiled-coil. This could explain why DivIB is essential only at high temperatures in B. subtilis (8, 19), if the (FtsL/DivIC) dimer of this species were stable enough at low temperature. This model could also explain why Campylobacter can divide without FtsQ, if the interaction between their FtsL and FtsB were strong enough. We propose to reverse our way of considering the interaction between FtsL and DivIC(FtsB). Instead of viewing the transmembrane segments and coiled-coil regions as a means of bringing together the C-terminal parts, the interaction of the C-terminal parts of FtsL and DivIC(FtsB) with DivIB(FtsQ) could help to zip together the coiled-coil helices and bring the transmembrane segments together.

We have seen no structuration of the α-domain upon binding of (KL/EC), which suggests the absence of interaction of the POTRA domain with the (FtsL/DivIC) dimer. No mutation affecting the recruitment of FtsL/DivIC(FtsB) to the division site was found in the POTRA domain of E. coli FtsQ (24). Also, the complete POTRA domain can be deleted from B. subtilis DivIB (23) without compromising growth. In contrast, mutations in the POTRA domains have been found to affect the recruitment of FtsQ and DivIB to the division site of E. coli and B. subtilis (23, 24). Could the POTRA domain intervene in the function of FtsL and DivIC(FtsB)? POTRA domain stands for polypeptide-transport-associated domain (25). Apart from DivIB(FtsQ), POTRA domains are found in β-barrel outer membrane proteins of Gram-negative bacteria, where they are thought to assist the folding of β-barrels by binding the unfolded polypeptide chain in an extended conformation (32). Coiled-coils, such as that found in FtsL and DivIC(FtsB), are assemblies of α-helices that are usually not folded individually prior to assembly. Thus, the extracellular region of FtsL and DivIC(FtsB) are likely to be unfolded without their partners (28). The POTRA domain could offer a binding interface, much as a chaperone, to FtsL and/or DivIC prior to their association. FtsL would be the preferred substrate of this chaperoning function of the POTRA domain, as it appears in two-hybrid experiments that FtsL alone interacts with FtsQ, whereas FtsB requires the presence of FtsL to interact with FtsQ (13). In these experiments, the interaction of FtsL might involve the POTRA domain, whereas the (FtsL/FtsB) dimer would interact with the β-domain of FtsQ.

The interaction of the β-domain of DivIB with the C-terminal regions of the FtsL/DivIC dimer revealed by our structural and interaction studies in vitro, opens new directions of investigation by suggesting that the coiled-coil region of FtsL and DivIC is free to interact with other partners, or plays a central role in itself.

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REFERENCES

1. den Blaauwen, T., de Pedro, M. A., Nguyen-Distèche, M., and Ayala, J. A. (2008) FEMS Microbiol. Rev. 32, 321–344
2. Harry, E., Monahan, L., and Thompson, L. (2006) Int. Rev. Cytol. 253, 27–94
3. Errington, J., Daniel, R. A., and Scheffers, D. J. (2003) Microbiol. Mol. Biol. Rev. 67, 52–65
4. Goehring, N. W., and Beckwith, J. (2005) Curr. Biol. 15, R514–R526
5. Aarsman, M. E., Piette, A., Frapoçt, C., Vinkenveugel, T. M., Nguyen-Distèche, M., and den Blaauwen, T. (2005) Mol. Microbiol. 55, 1631–1645
6. Buddelmeijer, N., Judson, N., Boyd, D., Mekalanos, J. J., and Beckwith, J. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 6316–6321
7. Ghigo, J. M., Weiss, D. S., Chen, J. C., Yarrow, J. C., and Beckwith, J. (1999) Mol. Microbiol. 31, 725–737
8. Daniel, R. A., and Errington, J. (2000) Mol. Microbiol. 36, 278–289
9. Katis, V. L., Wake, R. G., and Harry, E. J. (2000) J. Bacteriol. 182, 3607–3611
10. Buddelmeijer, N., and Beckwith, J. (2004) Mol. Microbiol. 52, 1315–1327
11. Noircrèc-Savoye, M., Le Gouëlec, A., Morlot, C., Dideberg, O., Vernet,
Structure of the DivIB-FtsL-DivIC Complex

T., and Zapun, A. (2005) 
Mol. Microbiol. 55, 413–424

12. Daniel, R. A., Noirot-Gros, M. F., Noirot, P., and Errington, J. (2006) 
J. Bacteriol. 188, 7396–7404

13. Karimova, G., Dautin, N., and Ladant, D. (2005) 
J. Bacteriol. 187, 2233–2243

14. Daniel, R. A., Williams, A. M., and Errington, J. (1996) 
J. Bacteriol. 178, 2343–2350

15. Guzman, L. M., Barondess, J. J., and Beckwith, J. (1992) 
J. Bacteriol. 174, 7716–7728

16. Levin, P. A., and Losick, R. (1994) 
J. Bacteriol. 176, 1451–1459

17. Le Gouëllec, A., Roux, L., Fadda, D., Massidda, O., Vernet, T., and Zapun, A. (2008) 
J. Bacteriol. 190, 4501–4511

18. Carson, M. J., Barondess, J., and Beckwith, J. (1991) 
J. Bacteriol. 173, 2187–2195

19. Beall, B., and Lutkenhaus, J. (1989) 
J. Bacteriol. 171, 6821–6834

20. Guzman, L. M., Weiss, D. S., and Beckwith, J. (1997) 
J. Bacteriol. 179, 5094–5103

21. Katis, V. L., and Wake, R. G. (1999) 
J. Bacteriol. 181, 2710–2718

22. Scheffers, D. J., Robichon, C., Haan, G. J., den Blaauwen, T., Koningstein, G., van Bloois, E., Beckwith, J., and Luirink, J. (2007) 
J. Bacteriol. 189, 7273–7280

23. Wadsworth, K. D., Rowland, S. L., Harry, E. J., and King, G. F. (2008) 
Mol. Microbiol. 67, 1143–1155

24. van den Ent, F., Vinkenvleugel, T. M., Ind, A., West, P., Veprintsev, D., Nanninga, N., den Blaauwen, T., and Löwe, J. (2008) 
Mol. Microbiol. 68, 110–123

25. Sánchez-Pulido, L., Devos, D., Genevrois, S., Vicente, M., and Valencia, A. (2003) 
Trends Biochem. Sci. 28, 523–526

26. Robson, S. A., and King, G. F. (2006) 
Proc. Natl. Acad. Sci. U.S.A. 103, 6700–6705

27. Gonzalez, M. D., and Beckwith, J. (2009) 
J. Bacteriol. 191, 2815–2825

28. Robson, S. A., Michie, K. A., Mackay, J. P., Harry, E., and King, G. F. (2002) 
Mol. Microbiol. 44, 663–674

29. Chao, H., Houston, M. E., Jr., Grothe, S., Kay, C. M., O’Connor-McCourt, M., Irvin, R. T., and Hodges, R. S. (1996) 
Biochemistry 35, 12175–12185

30. Jung, Y. S., and Zweckstetter, M. (2004) 
J. Biomol. NMR 30, 11–23

31. Lau, W. L., Ege, D. S., Lear, J. D., Hammer, D. A., and DeGrado, W. F. (2004) 
Biophys. J. 86, 272–284

32. Kim, S., Malinverni, J. C., Sliz, P., Silhavy, T. J., Harrison, S. C., and Kahne, D. (2007) 
Science 317, 961–964

33. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) 
Nucleic Acids Res. 22, 4673–4680

34. Ghosh, R., Egelhaaf, S. U., and Rennie, A. R. (2006) 
A Computing Guide for Small-angle Scattering Experiments, ILL Report No. ILL06GH05T, Institut Laue Langevin, Grenoble

35. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J., and Svergun, D. I. (2003) 
J. Appl. Crystallogr. 36, 1277–1282

36. Mylonas, E., and Svergun, D. I. (2007) 
J. Appl. Crystallogr. 40, S245–S249

37. Jacrot, B., and Zaccai, G. (1981) 
Ann. Physiol. 20, 2413–2426

38. Guinier, A. (1939) 
Biochemistry 35, 12175–12185

39. Semenyuk, A. V., and Svergun, D. I. (1991) 
J. Appl. Crystallogr. 24, 537–540

40. Svergun, D. I. (1999) 
Biophys. J. 76, 2879–2886

41. Konarev, P. V., Potoukhov, M. V., and Svergun, D. I. (2001) 
J. Appl. Crystallogr. 34, 527–532

42. Koradi, R., Billerter, M., and Wuthrich, K. (1996) 
J. Mol. Graph. 14, 51–55

43. Svergun, D. I., Richard, S., Koch, M. H., Sayers, Z., Kuprin, S., and Zaccai, G. (1998) 
Proc. Natl. Acad. Sci. U.S.A. 95, 2267–2272

44. Svergun, D. I., Barberato, C., and Koch, M. H. (1995) 
J. Appl. Crystallogr. 28, 768–773

45. Glatter, O. (1979) 
J. Appl. Crystallogr. 12, 166–175

46. Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. (2006) 
Bioinformatics 22, 195–201

47. Goehring, N. W., Petrovskaya, I., Boyd, D., and Beckwith, J. (2007) 
J. Bacteriol. 189, 633–645

48. Nyholm, T. K., Ozdirekcan, S., and Killian, J. A. (2007) 
Biochemistry 46, 1457–1465

49. Harrison, S. C. (2008) 
Nat. Struct. Mol. Biol. 15, 690–698