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Interaction between FtsZ and FtsW of *Mycobacterium tuberculosis**

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The recruitment of FtsZ to the septum and its subsequent interaction with other cell division proteins in a spatially and temporally controlled manner are the keys to bacterial cell division. In the present study, we have tested the hypothesis that FtsZ and FtsW of *Mycobacterium tuberculosis* could be binding partners. Using gel renaturation, pull-down, and solid-phase assays, we confirm that FtsZ and FtsW interact through their C-terminal tails, which carry extensions absent in their *Escherichia coli* counterparts. Crucial to these interactions is the cluster of aspartate residues Asp^{367} to Asp^{370} of FtsZ, which most likely interact with a cluster of positively charged residues in the C-terminal tail of FtsW. Mutations of the aspartate residues 367–370 showed that changing three aspartate residues to alanine resulted in complete loss of interaction. This is the first demonstration of the direct interaction between FtsZ and FtsW. We speculate that this interaction between FtsZ and FtsW could serve to anchor FtsZ to the membrane and link septum formation to peptidoglycan synthesis in *M. tuberculosis*. The findings assume particular significance in view of the global efforts to explore new targets in *M. tuberculosis* for chemotherapeutic intervention.

Crucial to bacterial cell division is the formation of the septum at midcell (1–3). During septation, FtsZ, a bacterial homolog of tubulin (4) based on its limited sequence similarity to tubulin, its binding and hydrolysis of GTP (5, 6), and its ability to form tubules, sheets, and minirings (7–9), localizes early at the division site to form the ring-shaped septum. Based on the crystal structure of the FtsZ from the archaeon *Methanococcus jannaschii*, the GTPase domain of FtsZ is located in the N-terminal portion of the molecule and is related to typical GTPases such as $p21^{ras}$ (10). The highly conserved N-terminal domain of *Escherichia coli* FtsZ extends up to residue 314 and contains all the elements required for nucleotide-dependent polymer formation. This region is followed by a region that is variable in length and sequence and extends up to residue 369 in *E. coli*. This variable region is followed by a 10-residue peptide that shows a high degree of sequence conservation and is called the C-terminal core domain. The C-terminal domain consists of a mainly parallel four-stranded β-sheet supported by two helices on one side. The C termini of FtsZ sequences are divergent. *Mycobacterium tuberculosis* FtsZ polymerizes more slowly than its *E. coli* counterpart (11), and its overexpression in *Mycobacterium smegmatis* leads to slow growth, clumping, and growth of branched filaments (12).

ftsZ is one of a number of genes required for cell division identified in *E. coli*. Other genes include *ftsA*, *ftsQ*, *ftsN*, *ftsL*, *ftsK*, *ftsW*, *ftsI*, and *zipA* (13). FtsW is a polytopic membrane protein that is present in virtually all bacteria that have a peptidoglycan cell wall (14, 15). It is required for cell division in *E. coli* (16, 17). Two functions have been attributed to FtsW: stabilization of the FtsZ cytokinetic ring (18) and facilitation of septal peptidoglycan synthesis by recruitment of FtsI (PBP3) to the division site (19). The first topological model of FtsW based on computational methods and experimental data has recently been proposed for the FtsW of *Streptococcus pneumoniae* (20). It features 10 membrane-spanning segments, a large extracytoplasmic loop, and both N and C termini located in the cytoplasm.

In *E. coli*, ZipA and FtsA interact with the C terminus of FtsZ. ZipA probably serves as the membrane anchor for FtsZ, and the interaction probably provides the driving force for cross-linking and clustering of FtsZ protofilaments (21). However, ZipA is found in only a small group of bacteria related to *E. coli.* FtsA is similar to actin (22). It may function by linking septum formation to peptidoglycan biosynthesis (23, 24). A search for new binding partners for the C terminus of FtsZ may provide insights into the mechanism of cell division in bacteria lacking ZipA and FtsA, which include the globally important pathogen, *M. tuberculosis*. The C termini of *M. tuberculosis* FtsZ and FtsW carry a string of amino acid residues that are absent in their *E. coli* counterparts. Clusters of oppositely charged residues at the C-terminal ends of FtsZ and FtsW raise the possibility that the cytoplasmic C-tail[1] of FtsW could possibly act as a membrane anchor for FtsZ. We have asked whether the C terminus of *M. tuberculosis* FtsZ interacts with the C terminus of *M. tuberculosis* FtsW. This report provides evidence that FtsZ and FtsW of *M. tuberculosis* are binding partners and that binding involves a cluster of aspartate residues in the C-tail of FtsZ. This is the first demonstration of the direct interaction between FtsZ and FtsW in any bacterium.

EXPERIMENTAL PROCEDURES

Molecular Biological Procedures—Standard procedures for cloning and analysis of DNA, PCR, electroporation, and transformation were used (25). Enzymes used to manipulate DNA were from Roche Molecular Biochemicals. DNA sequencing was performed using the Thermosequenase or the T7 Sequenase sequencing kits from Amersham Biosciences. All constructs made by PCR were sequenced to verify their integrity. The cosmid MTCY270 was a generous gift from Stewart Cole, Institut Pasteur, Paris, France. Kanamycin was used at a concentration of 50 μg/ml, and ampicillin was used at a concentration of 100 μg/ml.

The *ftsZ* gene was amplified from cosmid MTCY270 using the primer

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[1] The abbreviations used are: C-tail, C-terminal tail; GST, glutathione *S*-transferase; NBT/BCIP, nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate; Ni^{2+}-NTA, nickel-nitrilotriacetic acid; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PBS, phosphate-buffered saline; PBS-T, PBS containing 0.5% Tween.

TABLE I
Primers for amplification of derivatives of FtsZ

Primers for FtsZ derivatives		Mutation
Primer b	Primer c	
5′-AGGGCGGCACGTCGCAGCGTCGTCAT CAC-3′	5′-CGGCGGTGATGACGACGCTGTCGACGTGCCGCCCT-3′	D367A
5′-GCGGCACGTCGACGACAGCGGCGTCATCACCGCTG-3′	5′-CATCGGCGGTGATGACGCCGGTGTCGACGTGCCGC-3′	D367A, D368A
5′-CGTCGACAGCGGCGGCAGCACCGCCGATGCTCAGG-3′	5′-GAGCATCGGCGGTGATGCCGCCGCTGTCGACGTGC-3′	D367A, D368A, D369A
5′-CGTCGACAGCGGCGGCAGCACCGCCGATGCTCAGG-3′	5′-CCTGAGCATCGGCGGTGCTGCCCCCGCTGTCG-3′	D367A, D368A, D369A, D370A
5′-CCTGAGCATCGGCGGTGTCGACGTGCCGCCCT-3′	5′-AGGGCGGCACGTCGACACCGCCGATGCTCAGG-3′	Δ(D367 to D370)

Primer a

5′-GTTG**CTGCAG**ATGGGAGATGCCGCG-3′ (*Pst*I site in bold)

Primer d

5′-CT**ATCGAT**TGTATGGGAAGCCCG-3′ (*Cla*I site in bold)

pair, 5′-TATGGATC**CATATG**ACCCCCCCGCACAACTA-3′ (FtsZ sense) and 5′-TTTGT**CAATTG**CTCTCAGCGGCGCATGAAG-3′ (FtsZ antisense), with asymmetric *Nde*I and *Mun*I sites (in bold) and cloned between the *Nde*I and *Eco*RI sites of the vector pET28a (Novagen) to generate pJB101.

Mutants of FtsZ were generated by overlap extension PCR. The primers used are depicted in Table I. The initial rounds of PCR were carried out using the primer pairs *a* and *b*, and *c* and *d* and pJB101 as template. The products of each PCR were purified and used as templates for the second round of PCR using the primers *a* and *d*. The final products were cloned between the *Pst*I and *Cla*I sites of pJB101 to generate the mutants of *ftsz* in pET28a. FtsW was amplified from cosmid MTCY270 using the primer pair 5′-ATCGGATC**CATATG**CTA-ACCCGGTTGCTGC-3′ (FtsW-s) and 5′-CCC**GAATTC**ACCCGTAACG-CTGACCTTC-3′(FtsW-as) with asymmetric *Nde*I and *Eco*RI sites (in bold) and cloned between the *Nde*I and *Eco*RI sites of the vector pET28a to give pJB201.

The construct for expression of GST-FtsW(Ala^{490}-Gly^{524}) was generated using the sense primer 5′-TAT**GGATCC**ATGGCCGATCCGCCGG-TTCGT-3′ (*Bam*HI site in bold) and the antisense primer FtsW-as and cloning between the *Bam*HI and *Eco*RI sites of the vector pGEX-2T (Amersham Biosciences). To express the domain encompassed by residues Leu^{385} to Gly^{524} of FtsW, pJB201 was digested with *Pst*I and *Eco*RI, and the resulting 417-bp fragment was gel-purified and cloned between the *Pst*I and *Eco*RI sites of the vector pBAD-HisC (Invitrogen) to give pJB202.

Expression of FtsZ and Its Mutants—E. coli BL21(DE3)/pJB101 was grown to an A_{600} of 0.6. IPTG was added to a final concentration of 0.1 mM, and growth was continued at 37 °C with shaking for 2 h. Cells were harvested and broken by sonication in 10 mM Tris-HCl, pH 7.5, containing 1 mM $MgCl_2$, 1 μg/ml DNase, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin, and 0.1 mM phenylmethylsulfonylfluoride. The post-sonicate supernatant was loaded on a Ni^{2+}-NTA-agarose column equilibrated with 50 mM sodium phosphate, pH 7.4, containing 0.5 M NaCl, 1% (v/v) Triton X-100 (buffer A). After washing the column with 100 mM imidazole in buffer A, FtsZ was eluted with 200 mM imidazole in buffer A. The mutant FtsZ proteins were expressed as described above. All the mutants localized to the post-sonicate supernatant and were purified as described.

Biotinylation of FtsZ—Purified FtsZ (or [Δ(Asp^{367}–Asp^{370})]FtsZ)[2] was dialyzed against phosphate-buffered saline (PBS). After determining the concentration of FtsZ by the Bradford assay, 10-fold molar excess of *N*-hydroxysuccinimido-iminobiotin was added, incubated for 2 h at room temperature with occasional gentle mixing followed by dialysis against PBS.

Expression of FtsW and Its Derivatives—E. coli BL21(DE3)/pJB201 was grown to an A_{600} of 0.6. IPTG was added to a final concentration of 1 μM, and growth was continued at 25 °C for 6 h to express His-tagged FtsW. For expression of (Leu^{385}–Gly^{524})FtsW, *E. coli* Top10/pJB202 was grown to an A_{600} of 0.6, and arabinose was added at a concentration of 0.02% (w/v). Growth was continued for 4 h at 37 °C. GST-FtsW(Ala^{490}–Gly^{524}) was expressed by induction of cells with 0.1 mM IPTG at 37 °C for 2 h.

Blot Overlay Assay—Extracts from cells expressing FtsW were separated by SDS-PAGE, and proteins were electrophoretically transferred to nitrocellulose paper. The blot was blocked with blocking buffer (150 mM NaCl, 10 mM sodium phosphate, pH 7.5, 0.05% (v/v) Tween 20, containing 3% (w/v) gelatin) for 30 min at room temperature. The blotted proteins were allowed to renature in renaturing buffer (150 mM NaCl, 10 mM sodium phosphate, pH 7.5, 0.05% (v/v) Tween 20, 40 mg/ml bovine serum albumin) for 2 h at room temperature. After thorough washing with ligand blot buffer (150 mM NaCl, 10 mM sodium phosphate, pH 7.5, 0.05% (v/v) Tween 20), the blot was incubated with 1 μM biotinylated FtsZ (or [Δ(Asp^{367}–Asp^{370})]FtsZ) for 1 h at room temperature. As controls, similar blots were incubated in buffer alone without any FtsZ (or its mutant) for 1 h. After washing the blots with ligand buffer followed by incubation with streptavidin-linked alkaline phosphatase, detection was carried out using NBT/BCIP.

Pull-down Assay—GST-FtsW(Ala^{490}–Gly^{524}) was allowed to bind to glutathione-Sepharose. The slurry was washed thoroughly. Post-sonicate supernatants from *E. coli* cells expressing FtsZ or its mutants as N-terminal-hexahistidine-tagged proteins were incubated in each tube containing glutathione-Sepharose-bound GST-FtsW(Ala^{490}–Gly^{524}) for 2 h at 30 °C. The slurry was washed thoroughly with PBS, boiled in SDS gel sample denaturing buffer, and loaded on SDS-polyacrylamide (10%) gels. The separated proteins were electroblotted onto nitrocellulose, blocked in blocking buffer (as described above), and probed with anti-His antibody (Roche Molecular Biochemicals). Color development was carried out by incubation with anti-mouse IgG (alkaline phosphatase conjugate) and NBT/BCIP. Similar experiments were performed in which the post-sonicate supernatant from *E. coli* expressing GST-FtsW(Ala^{490}–Gly^{524}) was incubated with Ni^{2+}-NTA-agarose-bound His-tagged FtsZ or its derivatives. Detection of resin-bound GST-FtsW(Ala^{490}–Gly^{524}) was carried out using anti-GST antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Solid-phase Binding Assay for Studying the Interaction between FtsZ or Its Variants and FtsW—-GST-FtsW(Ala^{490}–Gly^{524}) was adsorbed to the wells of a microtiter enzyme-linked immunosorbent assay plate. Adsorption was allowed to proceed for 12 h at 4 °C. The wells were washed with PBS containing 0.5% Tween (PBS-T). Nonspecific binding sites were blocked with blocking buffer (1% (w/v) bovine serum albumin in PBS-T) for 1 h at 37 °C. The wells were washed extensively with PBS-T. 100 μl of biotinylated FtsZ or its mutants (0.2–10 μM) was added to each well, and the binding was allowed to proceed for 6 h at room temperature. The wells were then washed extensively with PBS-T. Binding of biotinylated protein was measured by adding 0.1 μg/ml (in 100 μl) streptavidin-alkaline phosphatase to each well, incubating for 60 min at room temperature followed by color development by the addition of 1 mg/ml *p*-nitrophenyl phosphate prepared in 0.1 M glycine containing 0.01 M $MgCl_2$ (pH 10.4). Absorbance was measured at 405 nm. In each case, experiments were performed in which equivalent amounts of bovine serum albumin were first adsorbed to the wells.

RESULTS

M. tuberculosis FtsZ Interacts with FtsW—The sequences of *M. tuberculosis* $H37R_v$ FtsZ (encoded by the open reading

[2] The nomenclature is as follows: [Δ(Asp^{367}–Asp^{370})]FtsZ refers to N-terminal His-tagged FtsZ with deletion of amino acid residues Asp^{367} to Asp^{370}; (Leu^{385}–Gly^{524})FtsW refers to amino acid residues Leu^{385} to Gly^{524} of carrying a His-tag at the N-terminus; GST-FtsW(Ala^{490}–Gly^{524}) refers to amino acid residues Ala^{490}–Gly^{525} of FtsW fused at the N-terminal end to GST; FtsZ(D367A) refers to FtsZ with aspartic acid residue at position 367 mutated to alanine, and so on.

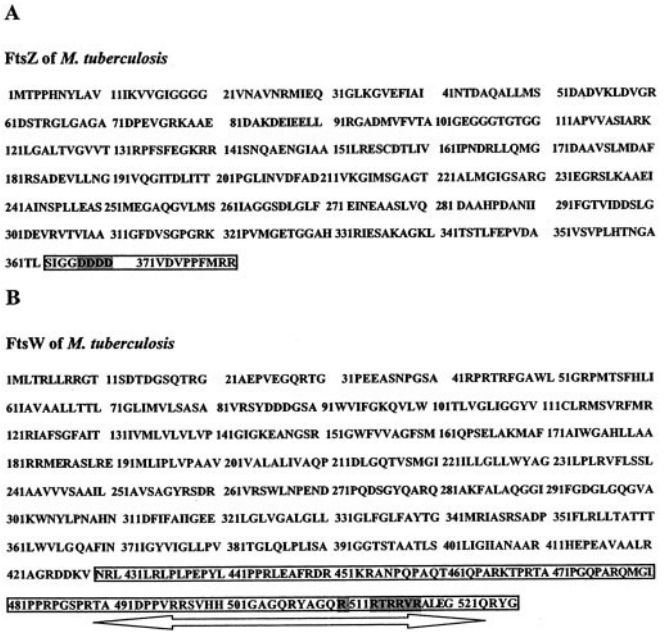

FIG. 1. *A*, deduced amino acid sequence of *M. tuberculosis* FtsZ. Amino acid residues 363–379 (*boxed*) represent the C-terminal extension that is absent in *E. coli* FtsZ. This includes a stretch of aspartate residues 367–370 (*shaded* in *gray*), which have been deleted or mutated successively to alanine in the various constructs used in the present study. *B*, deduced amino acid sequence of *M. tuberculosis* FtsW. Amino acid residues 428–524 (*boxed*) represent the C-terminal extension that is absent in *E. coli* FtsW. This includes a cluster of arginine residues between residues 510 and 516 (*shaded* in *gray*). The *double-headed arrow* indicates the stretch of residues from Ala^{490} to Gly^{525} expressed as a GST fusion protein.

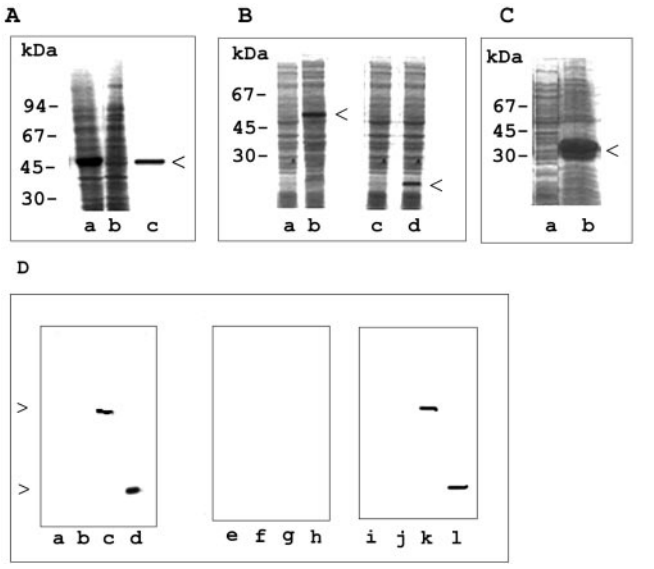

FIG. 2. **Expression of proteins and blot overlay assays.** Coomassie Blue-stained gels are shown. *A*, induced (*lane a*) and uninduced (*lane b*) *E. coli* cells expressing His-tagged FtsZ, and purified His-tagged FtsZ (*lane c*). *B*, uninduced (*lanes a* and *c*) and induced *E. coli* cells expressing His-tagged FtsW (*lane b*) and His-tagged (Leu^{385}–Gly^{524})FtsW (*lane d*). *C*, uninduced (*lane a*) and induced (*lane b*) *E. coli* cells expressing GST-FtsW(Ala^{490}–Gly^{524}). *Arrowheads* indicate positions of induced proteins. *D*, uninduced *E. coli*/pJB201 (*lanes a*, *e*, and *i*) or *E. coli*/pJB202 (*lanes b*, *f*, and *j*) or *E. coli* cells expressing His-tagged FtsW (*lanes c*, *g*, and *k*) or (Leu^{385}–Gly^{524})FtsW (*lanes d*, *h*, and *l*) were run on SDS-polyacrylamide gels, transferred to nitrocellulose, renatured, and incubated with biotinylated FtsZ (*lanes a–d*) or biotinylated [$\Delta(Asp^{367}$–$Asp^{370})$]FtsZ (*lanes e–h*) or anti-His antibody (*lanes i–l*) followed by incubation with streptavidin-alkaline phosphatase (*lanes a–h*) or anti-mouse IgG-alkaline phosphatase-conjugate (*lanes i–l*) and detection using NBT/BCIP.

frame Rv2150c)and FtsW (encoded by the open reading frame Rv2154c) are depicted in Fig. 1. The C-terminal extension of *M. tuberculosis* FtsW, which is absent in FtsW of *E. coli*, extends from amino acid residue 428 to residue 524 (Fig. 1*B*). A Kyte-Doolittle hydropathy plot (data not shown) suggests that this extension is hydrophilic. It encompasses a cluster of arginine residues (*shaded* in *gray*). The C-tail of FtsZ carries a stretch of aspartate residues (*shaded* in *gray*) that is absent in *E. coli* FtsZ (Fig. 1*A*). To test whether FtsZ interacts with FtsW, full-length FtsZ was expressed with a hexahistidine tag at its N terminus, purified on Ni^{2+}-NTA-agarose (Fig. 2*A*), and biotinylated. Efforts to purify FtsW did not meet with success. We failed to extract membrane-bound FtsW with detergents since the protein appeared to be sensitive to proteolytic degradation. Cell extracts expressing FtsW (Fig. 2*B*, *lanes a* and *b*) with a hexahistidine tag at the N terminus were separated by SDS-PAGE, electrotransferred on to nitrocellulose, and renatured. The blots were then incubated successively with biotinylated FtsZ and streptavidin-alkaline phosphatase followed by color development with NBT/BCIP. The reactivity of streptavidin-alkaline phosphatase alone was tested by incubation with blotted proteins in the absence of biotinylated FtsZ. A band developed at the position of migration of FtsW (Fig. 2*D*, *lane c*) The position of FtsW was verified by Western blot of a similar lane with anti-His antibody (Fig. 2*D*, *lane k*), suggesting a direct interaction between FtsZ and FtsW. A similar band was also obtained when an *E. coli* extract expressing (Leu^{385}–Gly^{524})FtsW (as an N-terminal His-tagged protein; Fig. 2*B*, *lanes c* and *d*) was used in similar assays. This domain includes the hydrophilic C-tail of FtsW, which is predicted to extend into the cytosol. (Leu^{385}–Gly^{524})FtsW could also interact with biotinylated FtsZ (Fig. 2*D*, *lane d*). These results suggested that the C-tail of FtsW is likely to be involved in a direct interaction between FtsZ and FtsW. We hypothesized that the stretch of aspartate residues (Asp^{367}–Asp^{370}) of FtsZ is involved in the interaction with FtsW (which possesses a stretch of arginine residues at its C-terminal end). A mutant of FtsZ in which these residues had been deleted was biotinylated and similarly tested for its ability to interact with FtsW. No interaction could be detected (Fig. 2*D*), leading to the conclusion that the stretch of aspartate residues at the C-tail of FtsZ is crucial for its interaction with FtsW.

Involvement of the C-tail of FtsZ in Interaction with the C-tail of FtsW in Vitro—To characterize further the interaction between the C-tails of FtsZ and FtsW, the C-tail of FtsZ and mutants where the aspartate residues had been successively mutated into alanine residues was expressed as N-terminal His-tagged fusion proteins and bound to Ni^{2+}-NTA-agarose. The hydrophilic C-tail of FtsW from residue Ala^{490} to Gly^{524} encompassing the cluster of arginine residues at the C terminus (Fig. 1*B*) was expressed as a GST fusion protein (Fig. 2*C*). Post-sonicate supernatant from *E. coli* cells expressing GST-FtsW(Ala^{490}–Gly^{524}) was incubated in separate tubes with immobilized FtsZ or its mutants, and resins were washed and boiled in SDS gel denaturing buffer. Interactions of GST-FtsW(Ala^{490}–Gly^{524}) with FtsZ or its mutants were analyzed after separation of the proteins on denaturing gels, electrotransfer, and Western blotting with anti-GST antibody. It was confirmed that His-tagged FtsZ could interact with the C-tail of FtsW (Fig. 3*A*). Mutation of one Asp to Ala did not abrogate the interaction. However, mutations of two aspartates to alanine led to a visibly diminished detectability of the band corresponding to GST-FtsW(Ala^{490}–Gly^{524}), whereas no band could be detected when three aspartates had been mutated to alanines. This led us to hypothesize that the stretch of aspartates located in the C-tail of FtsZ mediates its interaction with the C-tail of

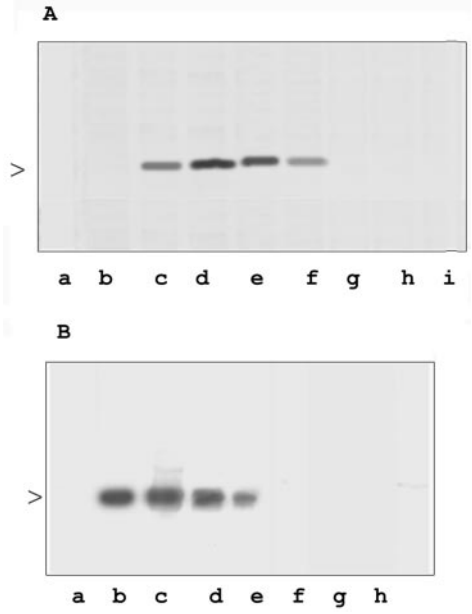

FIG. 3. **Pull-down assays.** *A*, cell lysates obtained from *E. coli* expressing His-tagged FtsZ (*lanes a*, *b*, and *d*), FtsZ(D367A) (*lane e*), FtsZ(D367A, D368A) (*lane f*), FtsZ(D367A, D368A, D369A) (*lane g*), FtsZ D367A, D368A, D369A, D370A) (*lane h*), or FtsZ(ΔD387 to D390) (*lane i*) were incubated with glutathione-Sepharose beads coupled to GST (*lane b*) or GST-FtsW(Ala^{490}–Gly^{524}) (*lanes d–i*). *Lane a* represents cell lysates obtained from *E. coli* expressing His-tagged FtsZ incubated with glutathione-Sepharose and run as a control for proteins (if any) adsorbed nonspecifically to the resin. The precipitates containing proteins bound to the Sepharose beads were analyzed by immunoblotting using anti-His antibody. The *arrowhead* indicates the position of His-tagged FtsZ. *Lane c* represents lysate from *E. coli* expressing His-tagged FtsZ. *B*, cell lysates obtained from *E. coli* expressing GST-FtsW(Ala^{490}–Gly^{524}) were incubated with Ni^{2+}-NTA resin alone (*lane a*) or resin bound to His-tagged FtsZ (*lane c*), FtsZ(D367A) (*lane d*), FtsZ(D367A, D368A) (*lane e*), FtsZ(D367A, D368A, D369A) (*lane f*), FtsZ(D367A, D368A, D369A, D370A) (*lane g*), or [Δ(Asp^{367}–Asp^{370})]FtsZ (*lane h*). The precipitates containing protein-bound resin were analyzed by immunoblotting with anti-GST antibody. The *arrowhead* indicates the position of GST-FtsW(Ala^{490}–Gly^{524}). *Lane b* represents lysate from *E. coli* expressing GST-FtsW(Ala^{490}–Gly^{524}).

FtsW with at least three aspartate residues being required for the interaction. These results were corroborated when GST-FtsW(Ala^{490}–Gly^{524}) was immobilized on glutathione-Sepharose, and post-sonicate supernatants of *E. coli* expressing FtsZ or its mutants were allowed to bind to it. FtsZ could not be pulled down in a control tube in which GST alone was bound to the resin, indicating that the interaction between the C-tail of FtsW and FtsZ was specific. After probing with anti-His antibody and color development, it was observed that the stretch of aspartate residues of FtsZ encompassed by residues 367–370 were involved in interaction with the C-tail of FtsW and that at least three aspartate residues were necessary for the interaction (Fig. 3*B*).

Analysis of the Interaction of FtsZ and the C-tail of FtsW by Solid-phase Binding Assay—Solid-phase binding assays were performed in which GST-FtsW(Ala^{490}–Gly^{524}) was adsorbed to the wells of a microtiter enzyme-linked immunosorbent assay plate, and varying concentrations of biotinylated FtsZ or its derivatives were added, followed by color development with streptavidin-alkaline phosphatase and *p*-nitrophenyl phosphate. Fig. 4 clearly shows that binding of FtsZ with the C-tail of FtsW was significantly diminished when two of the aspartate residues had been mutated to alanine. No detectable interaction was observed when three or more aspartate residues were changed to alanine.

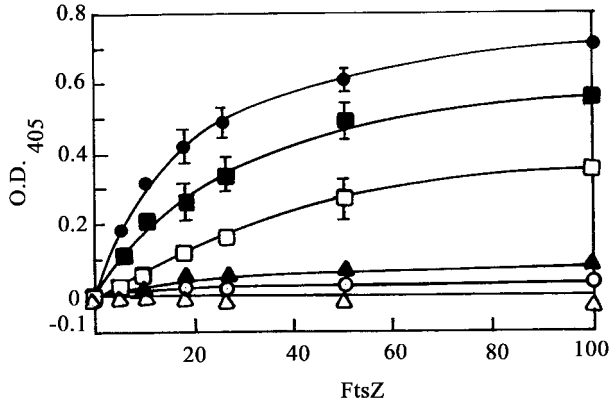

FIG. 4. **Solid-phase binding assay of the interaction of FtsZ with the C-tail of FtsW.** The interaction of biotinylated FtsZ or its mutants with GST-FtsW(Ala^{490}–Gly^{524}) was studied in microtiter plates as described under "Experimental Procedures." Each data point is the average of three determinations ± S.D. The *x* axis shows concentrations of FtsZ (or its derivatives) in μg/ml. FtsZ, (●—●); FtsZ(D367A), (■—■); FtsZ(D367A, D368A), (□—□); FtsZ(D367A, D368A, D369A), (▲—▲); FtsZ(D367A, D368A, D369A, D370A), (○—○); [Δ(Asp^{367}–Asp^{370})]FtsZ, (△—△).

DISCUSSION

Cell division is a complex multistep process requiring ordered assembly of a diverse array of proteins in a spatially and temporally controlled manner. Although the molecular events coordinating cell division have been best studied in *E. coli*, little is known about the cell duplication process of *M. tuberculosis*, a slow grower with a doubling time of 24 h. ZipA and FtsA have been identified as the interacting partners of FtsZ in *E. coli*. However, no counterparts of these two proteins have been identified in the *M. tuberculosis* genome. The mechanisms of cell division are likely to be different in *M. tuberculosis* as compared with *E. coli*. Learning about this process, which is restricted to the pathogen and absent in its host, is likely to enable the choice of rational targets for the development of potential new antimicrobial drugs. Cell division presents a case for developing drugs aimed at disrupting protein-protein interactions, as opposed to enzyme inhibitors. In the absence of ZipA, we have explored the possibility that FtsW could be the binding partner for FtsZ in *M. tuberculosis*, making it a likely candidate for linking septum formation to peptidoglycan biosynthesis. The C-terminal region of FtsZ is a domain important in mediating protein-protein interactions. We have observed that the C-tail of FtsZ carries a stretch of aspartate residues absent in its *E. coli* counterpart (Fig. 1*A*). The C-tail of FtsW of *M. tuberculosis* carries an extension not present in its *E. coli* counterpart. This C-tail is hydrophilic, predicted to extend into the cytosol, and carries a cluster of arginine residues (Fig. 1*B*). By analogy with the observation that arginine-rich clusters of the cytoplasmic domain of the human anion exchanger 1 serve as a major binding site for a motif of opposite charge in the membrane binding domain of protein 4.1 (26), we reasoned that the C-tails of FtsZ and FtsW could interact through the above mentioned clusters of oppositely charged residues. This hypothesis was tested using a range of biochemical techniques to characterize the interaction of the C-tails of FtsZ and FtsW. The results presented here validate our view that FtsZ and FtsW interact through their C-tails. Using a gel renaturation assay, we have demonstrated a direct interaction between FtsW of *M. tuberculosis* expressed in *E. coli* and biotinylated, recombinant FtsZ of *M. tuberculosis*. When the C-terminal domain of FtsW was expressed and used in gel renaturation assays, it was also able to interact with biotinylated FtsZ. This suggested that the C-tail of FtsW was likely to be crucial for

interaction with FtsZ. At the same time, [Δ(Asp^{367}–Asp^{370})]FtsZ was unable to interact with FtsW or its C-terminal domain, suggesting that the stretch of four aspartate residues in the C-tail of FtsZ is crucial for these interactions. Our predictions were further tested by mutating the aspartate residues one at a time and determining the minimum requirement of charged residues for an interaction between FtsZ and FtsW. Mutation of two aspartate residues led to a significant reduction in binding of FtsZ to FtsW, whereas no interaction was detectable when at least three of the aspartate residues had been mutated. Using immobilized GST-FtsW(Ala^{490}–Gly^{524}), pull-down assays showed that FtsZ could be precipitated from crude cell lysates, indicating that the interaction was specific. Pull-down occurred with progressively decreasing efficiency as the aspartate residues from positions 367 to 390 of FtsZ were mutated one after the other. These results strengthened the opinion that the string of aspartate residues from positions 367 to 370 was crucial in interacting with the C-tail of FtsW. Experiments in which lysates of *E. coli* expressing GST-FtsW(Ala^{490}–Gly^{524}) were allowed to interact with immobilized FtsZ or its mutants gave similar results. Taken together, these results provide, for the first time, evidence that FtsW of *M. tuberculosis* is an interacting partner of FtsZ from the same organism. The interactions most likely take place through the C-terminal ends of both these proteins, which carry clusters of oppositely charged residues. Survival of the pathogen within its host is likely to depend as much on protein-protein interactions as on enzymological functions. Protein-protein interactions that comprise large surface areas are unlikely to be suitable for targeted intervention. The FtsZ-FtsW interaction, on the other hand, appears likely to involve small clusters of charged residues on these proteins. The residues of FtsW involved in this interaction therefore deserve to be identified. Compounds that inhibit protein-protein interactions are far less well known than enzymological inhibitors. Taxol represents a chemotherapeutic that functions by stabilizing tubulin dimer associations (27, 28). As a prerequisite for developing chemotherapeutics, it is essential to characterize protein-protein interactions involved in key cellular processes such as cell division. Demonstration of the direct interaction of FtsZ and FtsW of *M. tuberculosis* marks a beginning. However, the results of the present study need to be interpreted with caution until it can be demonstrated that FtsW is indeed part of the division ring *in vivo*.

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