Genetic Analysis of the Escherichia coli FtsZ-ZipA Interaction in the Yeast Two-hybrid System

CHARACTERIZATION OF FtsZ RESIDUES ESSENTIAL FOR THE INTERACTIONS WITH ZipA
AND WITH FtsA

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Steven A. Haney‡§, Elizabeth Glasfeld‡, Cynthia Hale‡, David Keeney‡, Zhizhen He‡, and Piet de Boer‡
From the 2Department of Infectious Disease, Wyeth-Ayerst Research, Pearl River, New York 10965 and the 3Department
of Molecular Biology and Microbiology, Case Western Reserve University Medical School, Cleveland, Ohio 44106-4960

The recruitment of ZipA to the septum by FtsZ is an early, essential step in cell division in Escherichia coli. We have
used polymerase chain reaction-mediated random mutagenesis in the yeast two-hybrid system to analyze this
interaction and have identified residues within a highly conserved sequence at the C terminus of FtsZ as the ZipA
binding site. A search for suppressors of a mutation that causes a loss of interaction (ftsZD373G) identified eight
different changes at two residues within this sequence. In vitro, wild type FtsZ interacted with ZipA with a high
affinity in an enzyme-linked immunosorbent assay, whereas FtsZD373G failed to interact. Two mutant proteins
examined restored this interaction significantly. In vivo, the alleles tested are significantly more toxic than the wild
type ftsZ and cannot complement a deletion. We have shown that a fusion, which encodes the last 70 residues
of FtsZ in the two-hybrid system, is sufficient for the interaction with FtsA and ZipA. However, when the wild
type sequence is compared with one that encodes FtsZD373G, no interaction was seen with either protein.
Mutations surrounding Asp-373 differentially affected the interactions of FtsZ with ZipA and FtsA, indicating that
these proteins bind the C terminus of FtsZ differently.

In bacteria, the study of cell division has defined many genes acting in the formation and cleavage of a septum (1–3).
Currently, the earliest known step in the development of the septum is the formation of the Z-ring, a circular polymeric
structure formed by the tubulin-like protein FtsZ (4). The cell division machinery assembles on the Z-ring in a
sequential manner. Two proteins that act early in cell division, and directly on the Z-ring, are FtsA and ZipA (5–10).
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sequential manner. Two proteins that act early in cell division, and directly on the Z-ring, are FtsA and ZipA (5–10).
The structure of FtsA has been solved recently (11), and it supports previous indications that it is similar to actin (12, 13). FtsA may function by linking septum formation to peptidoglycan synthesis (8, 14). ZipA is an integral membrane protein that causes FtsZ fibers to bundle in vitro (15, 16). ZipA is thought to stabilize the FtsZ rings, in part because moderate overexpression of ZipA can
suppress the ftsZ84 allele, which confers a defect in Z-ring formation (16). The structure of the highly conserved C-terminal
fragment of ZipA has been solved by x-ray crystallography and NMR, and it shows conservation to several RNA-binding
proteins (17, 18).

Genetic analyses of cell division have identified many genes as conditional alleles, but detailed genetic analysis describing
an individual protein-protein interaction is difficult, because many interacting proteins are involved, often simultaneously
(19–21). Because of this, it is advantageous to develop a surrogate system that allows the study of a protein-protein inter-
action through genetic analysis (i.e. the study of a protein-protein interaction through the identification and char-
acterization of mutations). The two-hybrid system of yeast (Y2H) is one such system, because of the wide range of genetic
techniques that exist for yeast (22, 23) and because the two-hybrid system itself is a robust system for characterizing
protein-protein interactions in vivo (24). Thus, it is a relatively simple extension of yeast genetic analysis to examine a
two-hybrid interaction genetically. Previous work has indicated that the C terminus of FtsZ is important for the interaction
with both ZipA and FtsA (9, 10, 15, 21, 25). To better define the interaction between FtsZ and ZipA, we have analyzed this
interaction in yeast genetically. Using a mutation in ftsZ that reduces the interaction with zipA in the Y2H system, we
have searched for intragenic suppressors (additional mutations within ftsZ that reverse the phenotype of the initial loss-of
function mutation). This search has identified mutations within a small segment of ftsZ that encodes a conserved
sequence at the C terminus. We have characterized the effect of these mutations on the interactions with ZipA and FtsA and
determined the effect of the mutations on cell division.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Oligonucleotides—All strains and plasmids used in this study are listed in Table 1s. Oligonucleotides are listed in
Table 2s. Both tables are published as supplemental material in the online version of this paper.

Media and Reagents— Yeast and bacterial media were prepared by standard methods using materials readily available (22, 23). SC, YPD
and other general yeast media are described in these references. YNB, BactoAgar, BactoTryptone, BactoPeptone, and yeast extract
were purchased from Difco. Amino acid mixtures (CSM-LUTH, CSM-AHT), raffinose, glucose, and galactose were purchased from Bio101. Amino
acids, 5′-fluoroorotic acid (FOA), 4-methylumbelliferyl β-D-galactoside, and aminotriazole were purchased from Sigma Chemical Co. Zymolyase was purchased from ICN Biologics.

Construction of Plasmids—All oligonucleotides used in this study are listed in Table 2s. FtsZ and ZipA were cloned by PCR amplification of genetic regions from the plasmid pSH256 with oligos FtsZ-cally to generate plasmid pSH230. The plasmid pSH228 was consequently excised and cloned into pGAD424, which was digested identically in an ELISA. The lysine in this sequence is biotinylated in vitro by the E. coli enzyme BirA. Plasmids were constructed by inserting the coding sequence for the biotin tag between the NcoI and NdeI sites of pET28 (Novagen) using the oligos BIOTAGT and BIOTAGB. In addition, birA was amplified from the plasmid pBIOTRX-BirA (26) by PCR using the oligos BirA 5′ and BirA 3′, digested with HindIII and XhoI and ligated into the HindIII and XhoI sites of the same vector as the biotin tag. The resulting fragment was digested with NcoI and NdeI and cloned into pGAD424 that has been digested identically. The resulting fragment was digested with EcoRI and SalI and ligated into pLEXA, to generate plasmid pSH47. This fragment was subsequently excised and cloned into pGAD424, which was digested identically to generate plasmid pSH230. The plasmid pSH228 was constructed by PCR amplification of plasmid pSH256 with oligos FtsZ-5′ and FtsZ-3′. The resulting fragment was digested with EcoRI and SalI and cloned into pAS2-1. Plasmid pSH232 was constructed by subcloning the FtsZ-cally gene into pGAD424, which had been digested with EcoRI and SalI as well.

Construction of Yeast Strains—Strain SHy9 was generated by growing selection strain CG 1945 serially for two 10 ml overnight cultures, with about a 10′′ cell inoculum each, and plating on SC plates supplemented with 1% FOA, and colonies were allowed to grow for 5 days. Several colonies that were grown were checked for all phenotypes, including loss of glycerol, isopropyl-β-D-thiogalactopyranoside (IPTG) once the A600 of the culture was between 0.5 and 1.0. At the same time, t-biotin was added to a final concentration of 0.1 mM. Cells were incubated at 37 °C for another 2–3 h, centrifuged, and resuspended in buffer A (50 mM Tris, pH 7.9, 50 mM KCl, 1 mM EDTA, and 10% glycerol) and stored at −70 °C. The proteins were then purified according to a previous report (33).

Protein concentrations of biotin-FtsZ and its mutants was determined by measuring the displacement of 2′(4′-hydroxyazobenzene)benzoic acid from avidin. In short, 40 μl of protein sample or buffer was mixed with 360 μl of 0.5 mg/ml avidin and 0.3 mM 2′(4′-hydroxyazobenzene)benzoic acid in 100 mM sodium phosphate, 150 mM NaCl, pH 7.2. The decrease in absorbance at 500 nM was measured, and the concentration of biotin was determined as described according to Gill and von Hippel (34). The biotin-tagged FtsZs were between 50% and 75% biotinylated.

ZipA-(23–328) was overexpressed from the plasmid pDB348 in BL21(DE3)pLysS. Expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) once the A600 of the culture was between 0.5 and 1.0. At the same time, t-biotin was added to a final concentration of 0.1 mM. Cells were incubated at 37 °C for another 2–3 h, centrifuged, and resuspended in buffer A (50 mM Tris, pH 7.9, 50 mM KCl, 1 mM EDTA, and 10% glycerol) and stored at −70 °C. The proteins were then purified according to a previous report (33).

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mental conditions are described in the legends for Tables I and II.

β-Galactosidase Assay— Cultures to be tested were grown for 36 h in 5 ml of SC-Leu-Trp media. New cultures were inoculated with 100 ml of the overnight cultures, and the new cultures were grown for 16 h. Cells densities were between 0.8 and 1.0 A460 for these strains. Samples of these cultures were assayed for LacZ activity in quadruplicate, in a 96-well microtiter plate (100 μl per well). Mixtures were sampled with 100 μl of lysis buffer and substrate (40 μl of Promega cell lysis buffer, 40 μl of 0.125 mg/ml 4-methylumbelliferyl β-n-galactosidase (Sigma), and 20 μl of 10× β-galactosidase assay salts). Samples were incubated at 30 °C for 4–8 h with shaking and read on a Victor II fluorescence plate reader from Wallac. Fluorescence intensity increased with time, and after 8 h, negative control wells showed about 400 units, whereas positive control

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for the FtsA standard two-hybrid systems. When

ftsZ when studied in the yeast two-hybrid system. This observation

resulting in FtsZ

ments could be important for the FtsZ

ZipA interaction. The

ments introduced during cloning suggested that one of the mutations could be important for the FtsZ/ZipA interaction. The first change was N45D, which has been characterized by Wang et al. (35) as one that affects the GTPase activity of FtsZ. This change was discounted as the cause of the altered interaction with ZipA by several experiments (Ref. 15, and results not shown). This evidence includes the observation that ZipA binding to FtsZ is not affected by guanine nucleotides and that deletion analysis of FtsZ had already shown that the GTPase domain of FtsZ was not involved in binding to ZipA. The other mutation, D373G, resulted in a change in a highly conserved region of the C terminus of FtsZ (discussed below). At the time this project began, the role of this region in cell division had not been characterized. This mutation provided us with an opportunity to study how a mutation with diminished function could be used to characterize this interaction.

The lack of growth in the galactose system provided a clear strategy for determining which residues in FtsZ facilitate binding to ZipA, through the selection of intragenic suppressors. Selection of suppressors that restored the FtsZ/ZipA interaction was achieved by PCR mutagenesis of the whole ftsZ gene as cloned into pGAD424, using primers that annealed to the GAL4 activation domain fragment and the ADH terminator. The primers allowed ∼300-bp extensions to both ends of the ftsZ gene. These extensions provided regions of homology that allowed the PCR products to be cloned by recombination (Fig. 2A) (30). The PCR products were transformed directly into strain SHy63 (a derivative of the Gal-Y2H strain CG1945 that had been previously transformed with pAS2-1-zipA), with the pGADGH vector that had been linearized, and is therefore not stable in yeast unless it has been repaired. Repair could be achieved by homologous recombination with the ends of the PCR products containing portions of the GAL4 activation domain gene, and the ADH terminator. Transformation into strain SHy63 allowed the selection of recombinant plasmids expressing ftsZ alleles. Alleles interacting with the Gal4bd-zipA fusion were identified through the activation of the GAL1p-HIS3 reporter. 73 colonies grew on plates that lacked histidine and were supplemented with 1 mM aminotriazole, two of which are shown in Fig. 2B. Plasmids were recovered and restested in yeast, and 12 plasmids were purified that could be recovered, had normal restriction analysis patterns, and conferred plasmid-dependent phenotypes (histidine prototrophy only when introduced into a strain that carried pAS2-1-zipA). The ftsZ genes in these 12 plasmids were sequenced to identify any mutations. Eight of the 12 plasmids contain mutations in the conserved C terminus region. The remaining four plasmids do not contain mutations that result in amino acid changes in FtsZ are presumed to contain mutations that affect the copy number of the plasmid or the expression of the Gal4-FtsZ hybrid protein and have not been characterized further. The eight mutations identify five different residue changes from the original plasmid. Two mutations are reversions to the wild type asparagine residue at position 373, two mutations change the Asp-373 residue to serine, and one changes this glycine to cysteine. The remaining suppressors change the highly conserved proline residue at position 375 to leucine (twice) and to serine. These mutations are indicated in Fig. 3. The residues that comprise the C terminus of the E. coli FtsZ are shown. Residues in capital letters show conservation among FtsZ proteins from prokaryotes and plants. The mutations identified in yeast that are critical for the interaction of FtsZ with ZipA map within this sequence, and no mutations from other regions of ftsZ were identified, indicating that these residues may comprise the principal region of interaction with ZipA.

The suggestion that these mutations have significant effects on the interaction of FtsZ with ZipA was confirmed by introducing some of them into an unmutagenized pGAD424-ftsZ plasmid, and rechecking the phenotypes. Wild type ftsZ was compared with ftsZD373G, ftsZD373S, and ftsZD375L, FtsZL. We checked the interaction with zipA and ftsZ in the galactose Y2H system (Fig. 4). The latter interaction was examined, because other work has suggested that FtsA also interacts with FtsZ at its C terminus, and we were therefore interested in characterizing this interaction (10, 21, 35). As can be seen in Fig. 4, the FtsZ/FtsA interaction is also highly sensitive to mutations in the C terminus of FtsZ. All strains tested showed good growth on plates supplemented with histidine (Fig. 4A). The interaction of ZipA with FtsZ is best scored on plates

| Plasmid       | Colony forming units, temperature/ IPTG |
|--------------|----------------------------------------|
|              | 30°C/μM                               |
| Vector       | 787                                   |
| Wild type ftsZ| 729                                   |
| ftsZD373G    | 642                                   |
| ftsZD373S    | 605                                   |
| ftsZD375L    | 550                                   |

This evidence includes the observation that ZipA binding to FtsZ is not affected by guanine nucleotides and that deletion analysis of FtsZ had already shown that the GTPase domain of FtsZ was not involved in binding to ZipA. The other mutation, D373G, resulted in a change in a highly conserved region of the C terminus of FtsZ (discussed below). At the time this project began, the role of this region in cell division had not
A hybrid system is specific and sensitive to mutations in ftsZ suppressors were analyzed as well, FtsZD373S and FtsZD373G, indicating that the mutation has a significant impact on the interaction of these two proteins. Two otherwise well behaved, suggesting that the mutation has a significant impact on the interaction of these two proteins.

Two suppressors were analyzed as well, FtsZD373S and FtsZD373G, and were shown to have dissociation constants of 6.2 (± 0.9) μM and 1.3 (± 0.2) μM, respectively. Both proteins show greatly improved interactions with ZipA, although neither protein interacts with ZipA as well as wild type FtsZ.

Further characterization of the interaction between ZipA and FtsZ was performed in vitro with purified proteins. ZipA, (23–328), the soluble form of the protein, which lacks the N-terminal membrane-spanning domain, and FtsZ proteins, expressed from the alleles examined in Fig. 3, were purified from E. coli to homogeneity. The interactions were tested in an ELISA, as shown in Fig. 5. Wild type FtsZ shows a high affinity for ZipA, having an apparent dissociation constant of 0.19 (±0.009) μM. The affinity of the FtsZD373G mutant for ZipA was too low to be quantified in this assay, despite the protein being otherwise well behaved, indicating that the mutation has a significant impact on the interaction of these two proteins. Two suppressors were analyzed as well, FtsZD373S and FtsZD373G, P375L, and were shown to have dissociation constants of 6.2 (±

Plasmids were transformed to strain CH3 [dadR− trpE− trpA− trpA− recA− Tn10], and transformants were grown overnight at 37 °C in LB + Ap (50 μg/ml) + glucose (0.1%). Cultures were diluted 200-fold in LB + Ap (50 μg/ml) supplemented with the indicated concentration of IPTG, and growth was continued at 37 °C for 4–5 h until A600 = 0.8–1.0. Division phenotypes were determined phase microscopy. Plasmids used in this experiment are described in Table I.

| Plasmid          | Cell division phenotype, [IPTG] |
|------------------|---------------------------------|
| Vector           | WT                 |
| Wild type ftsZ   | WT/Min             |
| ftsZD373G        | WT/Min             |
| ftsZD373S        | WT/Min             |
| ftsZD373G,P375L  | WT/Min             |
| D373S WT/Min     | WT                 |
| D373G WT/Min     | WT                 |
| D373G,P375L WT/Min | WT                 |

**A**

![aad](image1)

**B**

![orf2](image2)

**Fig. 2.** Isolation of intragenic suppressors of the ftsZD373G mutation. A, mutations in ftsZ were generated by mutagenic PCR amplification of the ftsZD373G allele as a construct in pGAD424. Primers for the amplification were to sequences about 300 bp away from the multiple cloning site of the vector, allowing for cloning by in vivo recombination and expression of clones carrying mutations that restored the interaction of ftsZ with ZipA. B, growth phenotypes of pGADGH-ftsZ plasmids recovered from the screen. Plasmids were transformed into yeast strain CG1945, along with either plasmid pAS2-1 or plasmid pSH227 (pAS2-1-zipA), as indicated, grown overnight, and spotted onto the indicated plates.
ZipA Binding Site of FtsZ Identified by Intragenic Suppressors

**Fig. 3. Summary of mutations isolated by PCR mutagenesis in the two-hybrid system.** The FtsZ C terminus is shown as both the wild type sequence (top line), and with the D to G mutation that was encoded by the template DNA for this work (second line). Suppressors isolated from this DNA are indicated in the third line. Residues Asp-373 to Pro-375 that comprise the signature DIP sequence are underlined. FtsZ residues are numbered. Conserved residues are capitalized.

**FtsZ C terminus mutant**

| Wild type | Mutant: |
|-----------|---------|
| pQTAKEPDYLDIFEAFLRKQad | pQTAKEPDYLGIFEAFLRKQad |

**Suppressors:**

C S D L S

**Fig. 4. Interaction of intragenic suppressors of the **$ftsZ^{D373G}$** mutation with **zipA** and **ftsA** in the two-hybrid system.** Yeast diploid strains resulting from crosses of yeast strain SHy22 containing pAS2-1, pH227 (pAS2-1-zipA), or pH100 (pAS2-1-ftsA) with yeast strain SHy23 strains containing pGAD424-ftsZ-based plasmids as indicated in the figure. Diploid strains for testing are grown overnight and spotted, as described in Fig. 1, onto the plates indicated in the figure. Diploid strains resulting from crosses of yeast strain SHy22 containing pAS2-1-zipA strains containing pGAD424-ftsZ-based plasmids as indicated in Fig. 4, but are omitted from the figure for clarity. Duplicate spots represent overnight cultures of two independent transformants.

**Fig. 5. Determination of the dissociation constants for the binding of FtsZ and FtsZ mutants by ZipA.** The interaction was assayed as described under “Experimental Procedures,” and the data were fit by linear regression with a steady-state affinity model. Each data point is an average of three repeats.

**Fig. 6. zipA and ftsA interact with the C terminus of ftsZ in the yeast two-hybrid system.** Strains were constructed as described in Fig. 4 using strain CG1945 instead of strain SHy22 for the bait plasmids (pAS2-1, pSH228 (pAS2-1-ftsA-(311–383)), and pSH239 (pAS2-1-ftsZ$^{D373G}$-(311–383))). Prey plasmids (pGAD424, pSH230 (pGAD424-zipA), and pSH232 (pGAD424-ftsA)) were transformed into strain SHy23 to obtain strains for mating. Controls were performed as described in Fig. 4, but are omitted from the figure for clarity. Duplicate spots represent overnight cultures of two independent transformants.

Next, we sought to determine whether ZipA and FtsA interact with identical residues in the FtsZ C terminus. Eight additional residues within this conserved sequence were mutated individually to alanine. Strains expressing derivatives of pGAD424-ftsZ that have specific residues changed to alanine are indicated in Fig. 7. The strains were characterized for histidine prototrophy and for β-galactosidase activity, as shown in the figure. In this experiment, strain CG1945 was used for the bait plasmids. In these diploids, background growth of the strains containing pAS2-1-zipA were more comparable to that of the strains containing pAS2-1-ftsA, so only the SC-LHT plate is shown. Robust growth on plates containing 0.5 mM AT was only seen with the zipA/ftsZ interactions (data not shown).

**Phenotypic Consequences of Mutations That Change Residues in the FtsZ C Terminal**—The mutations described above were assayed in *E. coli* to determine whether there were any biological consequences associated with them. The mutations were introduced into pDR3, a plasmid that expresses ftsZ under the control of the lac promoter, and can complement a ftsZ deletion when induced by IPTG. Two assays were performed. In the first, ftsZ alleles expressed under a regulated promoter were characterized for their ability to complement an ftsZ deletion. The results are presented in Table I. pDR3 efficiently complements in the presence of IPTG, whereas none of the mutants can. Additional effects can be seen in Table II, where the expression of ftsZ from the same plasmids as those in Table I are examined for dominant effects. Expression of extrachromosomal ftsZ is toxic at high levels, as can be seen when a strain carrying pDR3 is exposed to high concentrations of IPTG, which expresses the plasmid copy of the FtsZ gene to high levels.

Dominant effects can be seen for all of the mutated alleles, with strong effects seen with the ftsZ$^{D373G,P375L}$ allele and the ftsZ$^{D373G,P375L}$ allele. In these cases, an Sep phenotype is seen at substantially lower concentrations of IPTG. The same is true for the ftsZ$^{D373S}$ allele, which has a more modest phenotype relative to the other mutations in this assay as well, but is still substantially more toxic than the wild type gene expressed on a plasmid. Thus, a comparison of the ftsZ alleles with the wild type ftsZ indicates that all of the mutations examined in Tables I and II show profound cell division defects.

**DISCUSSION**

Recently, the bacterial cell division proteins FtsZ, ZipA, and FtsA have been structurally characterized, as well as the ZipA/FtsZ interaction (11, 17, 18, 36). In addition to these structural characterizations, functional and biological studies, such as those described in this report, will allow us to understand...
stand how biologically important residues function in these interactions. Results presented here show that several residues within the conserved C terminus of FtsZ play essential roles in cell division, and minor changes within this sequence can have lethal consequences.

The results presented in this study that characterize the role of individual residues in the biological context of the Y2H system are in general agreement with the *in vitro* characterization of Mosyak et al. (17). One area where differences were seen is the first group of conserved residues: Asp-370, Tyr-371, and Leu-372. All three residues were shown to contribute important binding energy in the BIAcore system, but only the Y371A change was shown to be important in the Y2H system. The L372A change functioned well in the Y2H system, despite being the most important of the three *in vitro*. It is possible that the FtsZ binding pocket of ZipA can accommodate the D370A and L372A changes in the Y2H system more effectively than it can *in vitro* or that it can accommodate the Y371A change in the *in vitro* system better than in the Y2H system. Two mutations, L372A and R379A, affected the interaction with ftsA to a greater extent than the interaction with zipA. The severe effect of the R379A change is especially interesting, because structural data show that the arginine residue is solvent-exposed when bound by ZipA, which is consistent with the modest effect of the D373G mutation. The Asp-373 side chain contributes a hydrogen bond to the main chain of FtsZ, which helps define the transition from β-sheet structure to α-helix. The change to glycine introduces a significant structural change, including the removal of this critical hydrogen bond. The Pro-375 residue also plays a structural role in defining the ZipA binding site. The residues that directly interact with ZipA are interspersed through the sequence that includes the Asp-373 and Pro-375 side chains shown. The ZipA structure is shown in green. Numbers indicate residues of the FtsZ peptide. The hydrogen bond between Asp-373 and the main chain of FtsZ is indicated by the white dashed line. In the inset, the D373G and P375L mutations are modeled into the ZipA-FtsZ structure. Representations were done using the program RIBBONS (37).
Although none of the recent studies can conclude that the conserved C terminus residues of FtsZ comprise the complete target sequence for ZipA, it is certainly true that the region plays a critical role in the interaction with ZipA. The interaction of the FtsZ C terminus with ZipA has been characterized extensively (5, 9, 17, 18, 21). Additionally, the results of Fig. 6 indicate the C-terminal tail of FtsZ is sufficient for the interaction of FtsZ with both ZipA and FtsA. The portion of FtsZ encoded by the Y2H fusion plasmid in this figure consists of the last 72 residues of FtsZ. The interaction of ZipA with a MBP-FtsZ(311–383) fusion protein, with the wild type protein, and the 17-residue peptide are all similar, as measured in a BIAcore biosensor assay. Therefore, it is possible to conclude that the mutations recovered in this study were limited to the conserved residues of the FtsZ C terminus, because this region defines the principal ZipA interaction site. Thus, we have met a goal of this study, which was to use a random genetic approach to help define a protein–protein interaction.

The suppressors identified in this study provide information about the sensitivity of the cell division machinery to changes in the interactions of its components. The FtsZ(D373G) allele has profound effects on cell division and viability, as well as conferring on the mutant protein a greatly reduced affinity for ZipA and for FtsA. For these proteins, these results are consistent with the importance of their proper interaction with FtsZ. The suppressors show that these interactions are extremely sensitive. Neither of the alleles characterized in detail in this study (FtsZ(D373G, P375L) or FtsZ(D373S) complement a deletion. In the case of the double mutation, this is less informative about the relation between affinity for ZipA and function, because it shows a dramatically reduced affinity for FtsA, which complicates our determination of why this mutation fails to complement. The other allele, ftsZ(D373S), causes more modest changes on the interaction with ftsA. No difference from wild type could be seen between ftsA and ftsZ(D373S) by Y2H assay, indicating that any change is relatively minor. The data, including Y2H and the in vitro analysis in Fig. 5, suggests that it is more likely that this allele fails to complement because of altered interactions with zipA. Although it is possible that another protein also interacts with FtsZ at this site, the simplest conclusion to draw at this time is that these proteins cannot tolerate even moderate changes in their interactions.

If the interaction characterized in this study represents an important antibacterial target, it is necessary to show that the interaction between ZipA and FtsZ is not just essential but is very sensitive to interference. Although it is true that many protein–protein interactions are essential, their identification as promising antibacterial targets depends on the interaction being sensitive to interference. In some cases, inhibition of expression or activity by 50% can be lethal. For many essential genes, reducing expression by 95% or more can result in no observable defect. Several genes have been characterized that are functional when they have activity at 1%, or less, of their wild type levels. Classic examples include nonsense mutations, when their phenotypes can be alleviated by suppressor tRNAs (38, 39). The strength of a protein–protein interaction, or an activity, as a pharmaceutical target can be evaluated by such data. Specifically, if reducing a protein–protein interaction moderately (50–80%) has phenotypic consequences, then it could be regarded as a strong pharmaceutical target. If reducing the interaction by 100-fold or greater is required to inhibit growth, it may be problematic to find a drug that can achieve this level of inhibition through its specific activity and pharmacokinetics, in a true in vitro situation. One of the reasons that cell division is considered an important area of antibacterial research is that many of its steps are very tightly controlled, and are very sensitive to changes in expression levels. Cell division is sensitive to changes of 2- to 4-fold in the expression of ftsZ, zipA, and other genes (3, 6, 40). If the FtsZ-ZipA interaction itself is as sensitive, then this interaction has potential as a target. The results presented here argue that this is the case.

It is clear that the yeast two-hybrid system is a powerful system for the study of protein–protein interactions (24, 41–45). In this report, we have taken advantage of commonly used techniques for classical genetic analysis in yeast (i.e. the characterization of gene function through the identification of mutations) and applied them to a protein–protein interaction of E. coli. The need for such methods rests on the observation that many of its steps are very tightly controlled, and are very sensitive to changes in expression levels. Cell division is sensitive to changes of 2- to 4-fold in the expression of ftsZ, zipA, and other genes (3, 6, 40). If the FtsZ-ZipA interaction itself is as sensitive, then this interaction has potential as a target. The results presented here argue that this is the case.

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