Analysis of the Interaction of FtsZ with Itself, GTP, and FtsA

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FtsZ is an essential cell division protein in Escherichia coli (11) and Bacillus subtilis (6) and is widely conserved among prokaryotes (4, 29, 40). It functions during cell division by forming a ring, designated the Z ring, at the division site that directs cell division (1, 8; reviewed in reference 25). The dynamics of Z-ring formation during the cell division cycle led to the suggestion that the Z ring is a cytoskeletal element that is formed through a nucleation event and self-assembly of FtsZ (8, 24). In vitro, FtsZ has GTPase activity (14, 30, 34) and can assemble into filaments consistent with its suggested cytoskeletal function (16, 31). The cytoskeletal structures observed by using immunofluorescence (1, 2) and by utilizing an FtsZ-GFP tag (27) are also consistent with the model.

During cell division, FtsZ is likely to interact with many proteins, including itself. Assembly of FtsZ into filaments and two-dimensional (2-D) sheets indicates that there must be several sites for self-interaction (16, 31). At least one interaction must occur between subunits along one axis in a protofilament, and another must occur between subunits in two adjacent protofilaments in 2-D sheets. In addition, FtsZ may interact with other proteins to recruit them to the division site. One of these is FtsA, which has been shown to localize to the division site in an FtsZ-dependent manner (3, 27). Another is ZipA, which has been shown to localize to the division site and interact directly with FtsZ (20). It’s possible that other division proteins may also be recruited to the division site through interaction with FtsZ.

ftsZ genes from a variety of organisms have been sequenced, and the lengths of the deduced proteins vary from just over 320 amino acids to over 500 amino acids. This heterogeneity is due largely to differences at the carboxyl terminus (5, 28). FtsZ can be divided into two segments: a large N-terminal segment of approximately 320 residues that is highly conserved and a C-terminal segment that is of quite variable length and less well conserved. Between the E. coli and B. subtilis FtsZs, which are of similar lengths, there is no homology in the C-terminal 60 amino acids except at the extreme carboxyl terminus (5). This homology is not conserved in archaeobacterial FtsZs (4, 29, 40).

FtsZ contains a sequence motif, GGGTGTG, very similar to the tubulin signature motif, GGGTGGSG. This observation led to the discovery of its GTPase activity (14, 30). Although involvement of the tubulin signature motif in the GTPase activity of tubulin is speculative, there is evidence that the corresponding sequence in FtsZ plays an important role. A mutation altering this sequence in FtsZ to GGGAGTG results in loss of function in vivo and a loss of GTPase activity in vitro (30), whereas a mutation changing this sequence to SGGTGTTG results in temperature sensitivity in vivo and reduced GTPase activity (14, 34) but increased ATPase activity in vitro (35). Similar mutations have not been reported for tubulin.

In tubulin, the GTP binding site is unknown, due to a lack of structural information; however, attempts to identify the sequences that compose the site have been made by sequence comparisons and cross-linking studies involving GTP and its analogs. The latter studies have led to the identification of three different peptides from amino acids 3 to 19, 63 to 77, and 155 to 174 that can be cross-linked to the nucleotides (21, 23, 36). Sequence comparisons, especially with members of the GTPase superfamily, have led to the suggestion that regions 36). Sequence comparisons, especially with members of the GTPase superfamily, have led to the suggestion that regions from amino acids 103 to 109, 295 to 298, and 203 to 206 may be analogous to the conserved motifs involved in GTP binding and GTPase activity among GTPase superfamily members (37). However, direct testing of this suggestion by mutation did not confirm this possibility (17).

FtsZ and tubulin show additional sequence similarities beyond the tubulin signature motif (24). These similarities include two pairs of closely spaced and highly conserved aspartic acid and asparagine residues. The spacing between these two conserved pairs and the tubulin signature motif is very similar in FtsZ and tubulin. Interestingly, in the GTPase superfamily it is an aspartic acid residue, closely linked to an asparagine residue, that determines the nucleotide specificity through hydrogen bonding to the guanine ring (reviewed in reference 10).

In this study, we have used proteolysis and the yeast two-hybrid system (18) to try to delineate the region of FtsZ involved in interaction with guanine nucleotides and with itself. Having located the GTP/GDP binding site to a central region,
we have further explored the interaction by examining several mutations that specifically alter the conserved residues. We have also detected an interaction between FtsZ and FtsA.

MATERIALS AND METHODS

Bacterial strains and plasmids. W3110, XL-1-blue, and DH5α were routinely used as strains for cloning and protein expression. JLF101 [ftsZ(Sfz)] and JKD7-2(pKD3) were used for complementation (7, 11). The latter strain carries ftsZ::kan with ftsZ supplied by the temperature-sensitive replicon pKD3. pCXZ was used for expression of B. subtilis ftsZ (ftsZBs) as described previously (38). pFI18HE was used as an expression vector for various E. coli ftsZ derivatives (19). pC72 contained the first 320 codons of ftsZ cloned into pFI18HE. pBEFB was derived from pBEF0 by deleting the HindIII fragments (9). Two-hybrid system plasmids pGBT9 and pGAD424 were used (Clontech) with various derivatives constructed by PCR. Primers 5′-ATAAGATATCATG TGGAGGTTCCGAAAC and 5′-GATGAATCATTAGCCGGCTTATTTG were utilized for ftsZBs with pCXZ as a template. The resulting plasmids were designated pGAD424/BSZ and pGGBT9/BSZ, respectively. The primer design ensured that ftsZBs was in frame with both the GAL4 binding domain (BD) and the activating domain (AD) in these two plasmids. PCRs were performed by utilizing Taq polymerase in a COY Tempcycler II for 30 cycles with each cycle consisting of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C. Other plasmids, containing various portions of ftsZBs, were constructed with the same approach. These plasmids are listed in Table 1.

<table>
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<th>Plasmid</th>
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<th>Construct on plasmid (amino acids)</th>
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We have also detected an interaction between FtsZ and FtsA.

Analysis of the interaction of FtsZ with GTP by proteolysis. Sensitivity to protease digestion was used to explore the interaction between FtsZ and nucleotides. Initially, both FtsZEc and FtsZBs were examined; however, it was determined that FtsZBs was more readily protected from proteolytic digestion by nucleotides, presumably because of tighter nucleotide binding (data not shown). Therefore, FtsZBs was utilized in subsequent studies, and a diagram of this protein is shown in Fig. 1.

RESULTS

Analysis of the interaction of FtsZ with GTP by proteolysis. Sensitivity to protease digestion was used to explore the interaction between FtsZ and nucleotides. Initially, both FtsZEc and FtsZBs were examined; however, it was determined that FtsZBs was more readily protected from proteolytic digestion by nucleotides, presumably because of tighter nucleotide binding (data not shown). Therefore, FtsZBs was utilized in subsequent studies, and a diagram of this protein is shown in Fig. 1. Upon SDS-PAGE, the FtsZBs migrates with an apparent molecular mass of 45 kDa even though its calculated molecular mass is close to 40 kDa (5). In the absence of nucleotide, most of the FtsZBs was readily degraded by trypsin into small fragments of less than 15 kDa, although a band of approximately 40 kDa showed some resistance to proteolysis (Fig. 2). In the presence of GTP, trypsin-resistant fragments of 40 and 32 kDa were observed. Identical results were obtained with GDP, but other nucleotides, including ATP, CTP, UTP, and GMP, provided no protection from proteolysis (data not shown). This result is consistent with nucleotide binding studies done with FtsZEc.
which demonstrated that it bound GTP and GDP and that this binding could not be competed by other nucleotides (30).

N-terminal sequencing of the 40-kDa fragment revealed that it had an intact N terminus, indicating that it must have arisen by a cleavage near the carboxyl terminus removing 5 kDa of the variable segment. The 32-kDa fragment had an N terminus of GLGAGAN, indicating that it arose from the 40-kDa fragment by cleavage after arginine 67 (Fig. 1). The appearance and relative stability of these proteolytic products in the presence of GTP or GDP suggest that they bind the nucleotide. To test this directly, FtsZ was treated with trypsin in the presence of GTP and the resultant fragments were separated by SDS-PAGE and blotted onto nitrocellulose to test for GTP binding. The 40-kDa fragment bound GTP, whereas the 32-kDa fragment did not (Fig. 3).

One possible reason that the 32-kDa fragment did not bind GTP was that it could not renature on the membrane. To test this possibility, radioactive GTP of various concentrations and specific activities was mixed with FtsZ and then incubated with trypsin. Following incubation with trypsin, the sample was UV-irradiated to cross-link any bound [α-32P]GTP, and the FtsZ was analyzed by SDS-PAGE and autoradiography. The results show that [α-32P]GTP was cross-linked to fragments with molecular masses of 40 and 32 kDa (Fig. 4). The ability of the 32-kDa fragment to be cross-linked to GTP suggests that either this fragment alone binds GTP or the binding of GTP to the intact protein is not sufficiently disrupted by trypsin digestion to eliminate cross-linking. For instance, the N-terminal 7-kDa fragment could remain bound after trypsin digestion and play a role in the binding of GTP.

Additional protease sensitivity experiments were done with chymotrypsin. Incubation of FtsZ with chymotrypsin led to degradation of most of the FtsZ (Fig. 5). The addition of GTP resulted in protection but a more complex pattern than observed with trypsin. Bands that are present in the lane without GTP are enhanced in the presence of GTP. Also, some bands, including bands 4 and 5, appear unique to the lanes with GTP. These results, along with those obtained with trypsin, demonstrate that the FtsZ conformation in the absence of GTP is fairly similar to the nucleotide-bound form; however, the nucleotide-bound form is more resistant to proteolysis. Also, nucleotide binding must cause some conformational change giving rise to bands 4 and 5 with chymotrypsin and the 32-kDa band with trypsin.

Sequence analysis of bands 1 to 5 allowed their origin to be determined. Band 1 is a mixture of two fragments, one having an intact N terminus and a second starting from amino acid 5. Both of these fragments must have arisen by cleavage of a 5-kDa fragment from the carboxyl end, similar to the 40-kDa fragment seen with trypsin. Bands 2 and 3 arose from band 1 by an additional cleavage after amino acids 41 and 51, respectively. Band 4 has an intact N terminus and could arise from band 1 by a second C-terminal cleavage removing a total of 13 kDa from the carboxyl end of the full-length protein. Band 5 is
a mixture of two fragments with the same N termini as those in band 1, but it must be missing a few more amino acids from the carboxyl end than band 4. To determine if any of these proteolytic fragments could bind GTP, they were blotted onto nitrocellulose and tested for binding. The results in Fig. 6 show that the fragments in bands 1 and 5 bind GTP, indicating that the carboxyl-terminal 14 kDa of FtsZ is not required for GTP binding. The GTP binding to band 5 (but not band 4) was confirmed by running a longer gel that clearly separated these fragments (data not shown). These results indicate that the GTP binding site is within the first 26 to 31 kDa.

**Mutagenesis of conserved aspartic acid and asparagine residues.** The results of the proteolytic digestion experiments suggest that the GTP binding domain of FtsZ is located within a central core region that has limited sequence homology to eukaryotic tubulins. In addition to the glycine-rich tubulin signature motif, there are two aspartate and two asparagine residues that are highly conserved among FtsZs and tubulins, with similar spacing from the tubulin signature motif (Fig. 7) (24). These conserved residues include D158, N165, N207, and D209 in *E. coli* FtsZ and D197, N204, N247, and D249 in yeast β-tubulin (33). To assess the importance of these residues to FtsZ function, we introduced mutations into the *E. coli ftsZ* gene contained on M13. Subsequently, the mutated gene was introduced into a low-copy-number plasmid to test for complementation and also introduced into an expression vector so that the mutant protein could be overproduced and isolated.

Low-copy-number plasmids carrying either the D158-to-N (D158N) or the D158A mutation complemented the *ftsZ*84(Ts) mutation and were able to displace a plasmid carrying the wild-type *ftsZ* gene in a strain with the chromosomal copy of the *ftsZ* gene disrupted (Table 2). These results indicate that this aspartic acid residue is not essential for *ftsZ* function. Also, the FtsZD158A mutant protein displayed a level of GTPase activity higher than that of the wild type (Table 2).

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**FIG. 3.** GTP binding by trypsin fragments of FtsZ. FtsZ was digested with trypsin in the presence of 1 mM GTP (5 μg of FtsZ and 0.15 μg of trypsin in a reaction volume of 10 μl). The reaction was stopped, and the proteolytic fragments were separated by SDS-PAGE. The fragments were transferred to nitrocellulose, incubated with [α-32P]GTP, and autoradiographed. Lane 1, molecular mass markers (the same as in Fig. 2); lane 2, FtsZ without trypsin; lane 3, FtsZ treated with trypsin in the absence of GTP; and lane 4, FtsZ treated with trypsin in the presence of GTP.

**FIG. 4.** Cross-linking of GTP to trypsin fragments of FtsZ. FtsZ (5 μg/10 μl) was incubated with trypsin (0.15 μg/10 μl) in the presence of GTP of various concentrations and specific activities. Reactions were stopped by placing the mixture in the cold, and the mixture was exposed to UV light for cross-linking. Samples were subjected to SDS-PAGE and autoradiographed. Lane 1, molecular mass markers (same as in Fig. 2); lane 2, FtsZ and trypsin, with no other additions; lane 3, FtsZ, trypsin, and 1 mM [α-32P]GTP (0.5 Ci/mmol); lane 4, FtsZ, trypsin, and 0.1 mM [α-32P]GTP (5 Ci/mmol); lane 5, FtsZ, trypsin, and [α-32P]GTP (3,000 Ci/mmol); lane 6, FtsZ and [α-32P]GTP (3,000 Ci/mmol).

**FIG. 5.** GTP partially protects FtsZ from digestion with chymotrypsin. Purified FtsZ (5 μg/10 μl) was incubated with chymotrypsin (1.5 μg/10 μl) in the presence or absence of GTP, and the digestion was monitored by SDS-PAGE and Coomassie staining. Lane 1, molecular mass markers (same as in Fig. 2); lane 2, FtsZ; lanes 3 to 6, FtsZ plus chymotrypsin. The GTP concentrations were as follows: lane 3, 0; lane 4, 1 mM; lane 5, 0.1 mM; and lane 6, 0.01 mM.

**FIG. 6.** GTP binding by chymotrypsin fragments of FtsZ, assessed by treating FtsZ with chymotrypsin in the presence of GTP. Proteolytic fragments were separated by SDS-PAGE and blotted onto nitrocellulose, which was incubated with labelled GTP and autoradiographed. Lanes 1, molecular mass markers (same as in Fig. 2); lanes 2, FtsZ and chymotrypsin incubated without GTP; lanes 3, FtsZ and chymotrypsin incubated in the presence of 1 mM GTP; lanes 4, FtsZ alone; lanes 5, BSA.
The other three residues were essential for ftsZ function (Table 2). Plasmids carrying ftsZN165Y and ftsZN165D did not display ftsZ function in either of the complementation tests. The mutant proteins carrying these substitutions for asparagine 165 retained the ability to bind GTP; however, they displayed markedly reduced GTPase activity. Likewise, ftsZN207D and ftsZN209N were unable to complement ftsZ-deficient cells. The FtsZN207D and FtsZD209N proteins both bound GTP as well as the wild type, suggesting that the alterations did not dramatically affect overall conformation; however, they had little GTPase activity (Table 2).

Highly conserved asparagine and aspartic acid residues are present in the N-terminal 67 amino acids of FtsZ. Mutagenesis revealed that they are essential, since both ftsZD45N and ftsZN43D failed to complement the ftsZ mutants (Table 2). The corresponding mutant proteins bound GTP, indicating that these residues are not necessary for this activity (Table 2). FtsZN43D bound GTP more effectively than the wild type, indicating that this protein either refolded more efficiently on the membrane or had a higher affinity for GTP. However, it was shown that FtsZN43D did not display GTPase activity, but none displayed a significant amount (data not shown).

Interaction of FtsZ with itself. FtsZ probably interacts with itself to form the Z ring. To try to determine in more detail potential interacting sites, we exploited the yeast two-hybrid system. Previously, it was shown that FtsZEc has an activation domain, so it cannot be used as bait in the two-hybrid system (22). In contrast, FtsZEc does not have an activation domain, as revealed by its failure to turn the appropriate indicator strain blue on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) when fused to the BD of GAL4 (data not shown). Therefore, we used FtsZEc fused to the BD of GAL4 in combination with constructs containing FtsZEc or FtsZEc fused to the AD of GAL4 (22). Full-length constructs yielded blue colonies on X-Gal, indicating that an interaction between FtsZ molecules can be detected in this system (Fig. 8). It also demonstrates that this interaction is conserved between the E. coli and B. subtilis FtsZs despite the divergence in sequence between these genes. In fact, quantitative determination of the β-galactosidase units indicates that the interaction between the heterologous FtsZs is slightly stronger. To try to further delineate the site of self-interaction, deletions from both the N' and the C' ends of the B. subtilis ftsZ gene were made, and the truncated genes were fused to the BD of GAL4 and tested with a construct expressing either the AD-FtsZEc or the AD-FtsZEc fusion. The results of a series of deletions are summarized in Fig. 8. Removal of 56 amino acids from the carboxyl terminus of FtsZEc, which removes the nonconserved region of FtsZ, had no effect on the interaction with either of the FtsZs on the AD. However, with removal of an additional 71 amino acids, no interaction was detected. Removal of 28 amino acids from the N terminus of FtsZEc resulted in a decrease in the interaction with FtsZEc; however, with removal of 99 amino acids from the N terminus, no interaction could be detected. From these results, a site for interaction between FtsZs can be defined in the N-terminal conserved domain, probably occurring between amino acids 100 and 326.

Analysis of the activity of truncated derivatives of FtsZ. The above analysis of FtsZ indicated that the carboxyl-terminal 57 amino acids are not required for FtsZ-FtsZ interaction. In addition, the nucleotide binding studies indicated that the extreme carboxyl segments of FtsZ were not required for GTP binding. To examine this further, several truncated ftsZ genes with stop codons at positions 321, 277, 253, and 220 were constructed by PCR and cloned into the expression vector.
pJF118HE to give pJC72, pJC74, pJC71, and pJC73, respectively. The truncations were made with ftsZ Ec so that the resultant alleles could be tested for complementation. Initial attempts at cloning the PCR fragments were unsuccessful, raising the possibility that the basal level of expression was deleterious. In an attempt to overcome this possible problem, pBS58, which contains ftsQ, ftsA, and ftsZ and results in a four- to fivefold increase in FtsZ, was introduced into the cloning strain. Previous work has shown that raising the level of FtsZ can suppress inhibition of cell division and lethality caused by expression of an ftsZ gene missing the first 37 codons (41).

Using pBS58 allowed us to isolate all four clones. Subsequent retransformation and selection for just the expression vector allowed plasmids containing the three shorter truncations to be separated from pBS58. However, pBS58 was always present with pJC72, even though it was not selected, suggesting that FtsZ320 was particularly toxic. Induction of the truncated FtsZ320, even in the presence of pBS58, led to filamentation and cell death, indicating that this truncated FtsZ interfered with division (data not shown). Thus, the variable carboxyl segment is essential for FtsZ activity, and a truncated protein lacking this segment is a potent inhibitor of division.

To further analyze the truncated proteins and to assess their biochemical activity, they were overproduced. Overproduction of the three shorter FtsZs led to aggregation, as indicated by their appearance in the low-speed pellet. In contrast, FtsZ320 behaved like the wild-type FtsZ throughout purification and was readily purified. Further analysis showed that FtsZ320 bound GTP as well as the full-length protein (data not shown) and had a similar level of GTPase activity (Fig. 9A). The GTPase displayed a lag similar to that of the wild-type protein, and the lag decreased with increasing protein concentration, as previously reported for the wild-type protein (Fig. 9B) (14, 30).

The ability of FtsZ320 to polymerize was examined by utilizing conditions that have been found to promote the polymerization of FtsZ in the absence of promoting agents such as DEAE dextran (to be published elsewhere) (32). This polymerization can suppress inhibition of cell division and lethality caused by expression of an ftsZ gene missing the first 37 codons (41). Using the yeast two-hybrid system, we have found that GTP binds to the N-terminal two-thirds of FtsZ and itself, GTP, and FtsA. By exploiting sensitivity to proteases, we found that GTP binds to the N-terminal two-thirds of FtsZ and can be cross-linked to a core region consisting of residues 67 to approximately 250. Using the yeast two-hybrid system, we have

![Diagram showing the inserts that were utilized in the yeast two-hybrid system to map the regions of FtsZ responsible for the interaction of FtsZ with itself and with FtsA. Interactions are indicated on the right, based on the results of color development on indicator plates. +++, color development within 1 h; +++, color development within 3 h; +, no color development within 24 h. Quantitation of the ß-galactosidase activity gave the following values for the full-length proteins: FtsZBs-FtsZBs, 8.5 U, FtsZBs-FtsZEc, 12 U; FtsA Bs-FtsZBs, 14 U; and FtsA Bs-FtsZEc, 10 U. These interactions are sufficient to yield a blue color in 30 to 60 min.](http://jb.asm.org/)

**Interaction**

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FtsZBs (pGAD or pGAD424)

**DISCUSSION**

In this study, we have examined the interaction of FtsZ with itself, GTP, and FtsA. By exploiting sensitivity to proteases, we found that GTP binds to the N-terminal two-thirds of FtsZ and can be cross-linked to a core region consisting of residues 67 to approximately 250. Using the yeast two-hybrid system, we have
detected an FtsZ-FtsZ self-interaction that is within the N-terminal conserved domain. Furthermore, interaction between FtsZ and FtsA was also detected by using the yeast two-hybrid system. Interestingly, the interaction of FtsZ with itself and with FtsA could also be detected between proteins from the evolutionarily distant species *E. coli* and *B. subtilis*, arguing that it is specified by conserved residues.

In the GTPase superfamily, the nucleotide specificity is determined largely by the sequence NKXD, in which the aspartate residue hydrogen bonds to the guanine ring (10). The FtsZ family contains three pairs of highly conserved aspartate and asparagine residues, two of which can be aligned with conserved residues in tubulin. To explore the interaction of FtsZ with GTP, we first looked at nucleotide protection from proteolysis. The presence of GTP or GDP protected a large, N-terminal, 40-kDa fragment of the *B. subtilis* FtsZ from proteolytic digestion. The carboxyl-terminal segment, which is quite variable among evolutionarily distant bacteria, is rapidly removed by proteolysis and is not required for GTP binding.

We have observed this proteolytic sensitivity with several proteases and several different bacterial FtsZs (data not shown), suggesting that the carboxyl tail is quite exposed, even in the presence of bound nucleotide. Furthermore, a truncated FtsZ lacking this variable sequence, FtsZ320, bound GTP and had GTPase activity similar to that of the full-length protein. Thus, the variable carboxyl tail is not needed for GTPase activity. The chymotrypsin experiments extend the carboxyl region that is dispensable for GTP binding to 13 kDa. The observation that GTP could be cross-linked to a 32-kDa fragment that lacked approximately 50 amino acids from the carboxyl end and 67 amino acids from the N terminus suggests that at least part of the binding site is within this fragment.

To examine the importance of the three ND pairs in GTP binding and FtsZ function, they were altered by site-directed mutagenesis. The four residues that are in the same relative positions in FtsZ and tubulin (Fig. 7) were not required for GTP binding. D158 was changed to aspartic acid and to alanine. Both of these mutant alleles complemented strains deficient in *ftsZ*, and the D158A protein had enhanced GTPase activity. These studies rule out a critical role for this residue in
ftsZ function. In contrast, the other three residues are critical for function. The N165Y and N165D mutations inactivated the gene, and the corresponding proteins had reduced GTPase activity. Likewise, the N207D and D209N mutant proteins lost ftsZ activity in vivo and had no GTPase activity. However, all of these mutant proteins bound GTP (binding by N165D was weak, whereas N165Y binding was similar to that of the wild type), suggesting that their conformation was largely intact and that these aspartic acid and asparagine residues are not playing a direct role in determining nucleotide specificity. The same is true for another aspartic acid residue conserved among FtsZs, D212, which is changed to a G by the ftsZ2 mutation (7). That mutant protein also binds GTP (13). An additional ND pair that is located nearer the N terminus and is also conserved among FtsZs was found to be essential for growth but not required for GTP binding. D45N and N43D mutant proteins both bound GTP but had reduced GTPase activity. Together, these results indicate that the determinants for the specificity of GTP binding by FtsZ have to be quite different than for the GTPase superfamily.

In vitro, FtsZ is able to polymerize in the presence of GTP to form protofilaments (16, 31). Since these protofilaments can align into a 2-D crystalline array, this suggests that FtsZ has to have several sites, both lateral and longitudinal, for it to interact with itself. By using the yeast two-hybrid system, we can detect at least one of these interactions in the FtsZcarboxy terminus. This interaction is also seen between the E. coli and B. subtilis FtsZs, and analysis of truncated derivatives indicates that the site can be defined between residues 100 and 326.

We also used the yeast two-hybrid system to demonstrate an interaction between FtsA and FtsZ. Interaction between these proteins would explain how FtsA is recruited to the Z ring during cell division (3, 27). This interaction did not require the first 59 amino acids of FtsZ but did require the nonconserved carboxyl tail. The latter finding is surprising, since the FtsA-FtsZ interaction was also observed between FtsAcarboxy and FtsZcarboxy, indicating that the interaction was between conserved residues. The only conserved residue in the carboxyl tail is at the extreme carboxyl end, which must play some role in the interaction (Fig. 1).

Evolutionarily distant FtsZs cause morphological abnormalities when introduced into E. coli. In most cases, this is a block to division (5, 28, 39, 40). The same appears to be true for FtsA, since B. subtilis ftsA is toxic to E. coli (28). From the results presented here, it is clear that the B. subtilis FtsZ and FtsA proteins are able to interact with FtsZcarboxy. Therefore, the B. subtilis proteins interact with the division machinery of the E. coli host but may be missing some function. The heterologous FtsZ may be equivalent to FtsZ320, which is a potent inhibitor of division. Recently, Ma et al. (27) have shown that introduction of a Rhizobium melleloli FtsZ-GFP fusion protein into E. coli results in the formation of cytoskeleton-like structures that suggest colocalization with the host FtsZ into nonfunctional structures.

Biochemical analysis of FtsZ320 complements the other studies reported here. It confirms that the variable C-terminal region is not required for GTP binding and revealed that it is not required for GTPase activity or polymerization. On the other hand, genetic studies demonstrate that this region cannot substitute for full-length FtsZ and that FtsZ320 is a potent inhibitor of cell division. This inhibitory activity is likely to arise from FtsZ320 copolymerizing with full-length FtsZ but failing in some subsequent step. Ma et al. (27) found that a similarly truncated FtsZ fused to GFP resulted in the appearance of unusual cytoskeletal structures, including spirals and thick sheets. The latter is consistent with our in vitro observations of the polymers formed by FtsZ320. Ma et al. also found that this fusion protein was more inhibitory to division than the full-length fusion protein. This suggests that copolymers formed between FtsZ and FtsZ320 are not capable of functioning in septation. Also, the two-hybrid system indicates that the C-terminally truncated FtsZ does not interact with FtsA. Together, these results indicate that the nonconserved carboxyl tail is on the surface of the filaments, where it would be available to interact with other proteins such as FtsA.

REFERENCES


