

Vinblastine induces an interaction between FtsZ and tubulin in mammalian cells

Xuan-chuan Yu¹, William Margolin¹, Manuel L. Gonzalez-Garay² and Fernando Cabral^{2,*}

¹Department of Microbiology and Molecular Genetics and ²Department of Integrative Biology, Pharmacology and Physiology, University of Texas Medical School, Houston, Texas 77030, USA

*Author for correspondence (e-mail: fcabral@farmr1.med.uth.tmc.edu)

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SUMMARY

The *Escherichia coli* cell division protein FtsZ was expressed in Chinese hamster ovary cells, where it formed a striking array of dots that were independent of the mammalian cytoskeleton. Although FtsZ appears to be a bacterial homolog of tubulin, its expression had no detectable effects on the microtubule network or cell growth. However, treatment of the cells with vinblastine at concentrations that caused microtubule disassembly rapidly induced a network of FtsZ filaments that grew from and connected the dots, suggesting that the dots are an active storage form of FtsZ. Cells producing FtsZ also exhibited vinblastine- and calcium-resistant tubulin

polymers that colocalized with the FtsZ network. The FtsZ polymers could be selectively disassembled, indicating that the two proteins were not copolymerized. The vinblastine effects were readily reversible by washing out the drug or by treating the cells with the vinblastine competitor, maytansine. These results demonstrate that FtsZ assembly can occur in the absence of bacterial chaperones or cofactors, that FtsZ and tubulin do not copolymerize, and that tubulin-vinblastine complexes have an enhanced ability to interact with FtsZ.

Key words: FtsZ, Tubulin, Cytoskeleton, Vinblastine, Microtubule

INTRODUCTION

FtsZ is a key protein in bacterial cell division (Bramhill, 1997; Erickson, 1997; Lutkenhaus and Addinall, 1997; Rothfield and Justice, 1997). It assembles into a ring at the site of bacterial cell division, and remains associated with the inner face of the cytoplasmic membrane throughout the septation process (Bi and Lutkenhaus, 1991; Sun and Margolin, 1998). Homologs of *ftsZ* have been found in every free-living prokaryote examined to date, including many species of archaea (Faguy and Doolittle, 1998), and in higher plants, where it is involved in chloroplast division (Osteryoung et al., 1998; Strepp et al., 1998). Although other cell division genes in chloroplasts and the archaea have not been characterized, eubacteria, with the exception of the wall-less Mycoplasmas, contain a large group of additional genes known to be involved in septation. In bacteria, such as *Escherichia coli* and *Bacillus subtilis*, there is good evidence that FtsZ directly recruits these other septation proteins to a ring complex at the site of division. Even in species with no other known homologs of cell division proteins, such as the archaeon *Haloferax volcanii*, FtsZ forms a ring at midcell (Wang and Lutkenhaus, 1996). Moreover, overproduction of FtsZ in the related archaeon *Halobacterium salinarum* results in the loss of normal rod shape (Margolin et al., 1996). Thus, FtsZ can be thought of as an essential cytoskeletal organizer for prokaryotes (Margolin, 1998).

FtsZ shares several properties with the eukaryotic

cytoskeletal protein tubulin, and is likely to be the ancestral tubulin homolog based on striking structural similarities (Löwe and Amos, 1998; Nogales et al., 1998). Like tubulin, FtsZ binds and hydrolyzes GTP (de Boer et al., 1992; Mukherjee et al., 1993; Raychaudhuri and Park, 1992) and forms dynamic, GTP-dependent polymers with a lattice structure similar to that of microtubules (Erickson et al., 1996; Lu et al., 1998; Mukherjee and Lutkenhaus, 1998; Yu and Margolin, 1997). To date, however, there is no convincing evidence that FtsZ and tubulin are able to interact in any meaningful way.

The assembly of protofilament bundles of FtsZ is stimulated and stabilized by DEAE-dextran or by millimolar Ca²⁺ (Erickson et al., 1996; Yu and Margolin, 1997). This effect of Ca²⁺ is notable because Ca²⁺ strongly inhibits tubulin assembly into microtubules (Weisenberg, 1972). In the case of Ca²⁺-promoted FtsZ assembly in vitro, filament bundles form from small dispersed aggregates, generating astral polymers that eventually interconnect laterally with polymers from other aggregates to create a network (Yu and Margolin, 1997). This process appears to mimic ring formation in *E. coli* cells; i.e. FtsZ appears to nucleate from a single site on the cell surface and rapidly polymerizes outward in opposite directions, forming a closed ring once the two growing polymers meet (Addinall and Lutkenhaus, 1996; Sun and Margolin, 1998). The most obvious difference between the two processes is that FtsZ assembly in vivo follows a path restricted to bidirectional polymerization in the plane of the ring, whereas polymers

made *in vitro* are free to grow outward from the nucleation center in any direction. Such astral polymers growing from a nucleation center are reminiscent of microtubules in animal and fungal cells that emerge from microtubule organizing centers. The structural and functional similarities between FtsZ and tubulin pose fundamental questions regarding the ability of FtsZ to assemble into polymers in eukaryotic cells, to nucleate from the eukaryotic centrosome, and to coassemble with or to disrupt microtubules.

To address these issues, we expressed *E. coli* FtsZ and its green fluorescent protein (GFP)-tagged version in Chinese hamster ovary (CHO) cells, and show that FtsZ often forms punctate accumulations or 'dots'. Importantly, we demonstrate that FtsZ does not interact with microtubules or disrupt their assembly under normal conditions. We then show that FtsZ can be induced to form polymers that grow from and connect the dots, by treating the cells with concentrations of vinblastine that normally cause microtubule depolymerization. The progression from localized nucleation centers to polymerization is similar to FtsZ assembly that has been observed *in vitro* as well as in bacterial cells. Finally, we show that vinblastine- and calcium-resistant tubulin polymers appear only in cells with FtsZ polymers, and that the tubulin and FtsZ polymers are coaligned. These results demonstrate that FtsZ and tubulin do not interact under normal conditions, but can be made to interact following drug treatment.

MATERIALS AND METHODS

Expression of FtsZ in mammalian cells

The *E. coli ftsZ* gene, lacking its ribosome-binding site (rbs), was cloned into pTOP_{neo}, a modified tetracycline-regulated mammalian expression plasmid that is described in detail elsewhere (Gonzalez-Garay et al., 1999). The *ftsZ* coding region was isolated from plasmid pGZ, a derivative of pBC SK (Stratagene, La Jolla, CA), which carries *lacZ*-GFP on a *Bam*HI fragment that is fused to the initiation codon of *ftsZ* and hence uses the *lac* rbs for expression of the fusion (Ma et al., 1996). The *ftsZ* segment in pGZ had been inserted downstream of GFP by cleaving a PCR product synthesized with primers ZEC1A (5'GGATCGATGTTTGAACCAATGGAAC3') and ZEC2A (5'GGGGGCCCTAATCAGCTTGCTTACG3') with *Cla*I and *Apa*I. The rbs-deficient *ftsZ* was then isolated as a *Hind*III-*Apa*I fragment and cloned into *Hind*III-*Apa*I-cleaved pTOP_{neo}. Removal of the *ftsZ* rbs was found to be essential in order to clone the gene into this plasmid. Initial attempts to clone *ftsZ* with its rbs failed, presumably because an upstream vector promoter could drive expression to toxic levels in bacteria.

Transformants in *E. coli* JM105 were selected on LB + 50 µg/ml ampicillin, and clones containing the intact *ftsZ* cDNA were designated WM726. Plasmid DNA was then purified using a QIAfilter Plasmid Maxi Kit (Qiagen Inc., Santa Clarita, CA) according to the manufacturer's instructions, and transfected into CHO strain tApuro 6.6 that expresses the tetracycline transactivator under tetracycline control (Gonzalez-Garay et al., 1999). Transfection was mediated by Lipofectamine (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions, and stably transfected cell lines were selected in medium containing 2 mg/ml G418 (Gibco BRL) and 1 µg/ml tetracycline (Sigma Chemical Co., St Louis, MO). For expression of an FtsZ-GFP fusion protein, a sequence encoding the m2 variant of GFP (Cormack et al., 1996) was added at the 3' end of the *ftsZ* coding sequence to create plasmid WM836. This was done by cleaving plasmid DNA from WM726 with *Bst*EII and *Apa*I, which removed the C terminus of FtsZ, and replacing it with the *Bst*EII-*Apa*I

fragment of pZG containing the same C terminus of FtsZ fused with GFP (Ma et al., 1996).

Growth of cells

Cells were maintained at 37°C and 5% CO₂ in alpha modification of minimal essential medium (α-MEM) supplemented with 50 i.u./ml penicillin, 50 µg/ml streptomycin and 5% fetal bovine serum (all from Gibco BRL). Medium for transfected cells additionally contained 2 mg/ml of G418 and 1 µg/ml tetracycline to ensure retention of the transfected plasmid DNA and to prevent expression of potentially toxic FtsZ. Induction of FtsZ production was carried out by culturing cells overnight (20 hours) in medium without tetracycline. Our studies indicate that protein production is detectable approximately 3 hours after tetracycline removal and reaches steady state by 16 hours (M. L. Gonzalez-Garay and F. Cabral, unpublished observations).

Fluorescence microscopy

For single- or double-label immunofluorescence, CHO cells were grown on glass coverslips to about 70% confluence, rinsed with PBS, and fixed for at least 10 minutes in methanol at -20°C. The cells were then rehydrated in PBS and incubated with affinity purified polyclonal anti-FtsZ (Yu et al., 1998) or monoclonal anti-α-tubulin (DM1A, Sigma) for 1 hour at 37°C in a humid chamber. Following several washes in PBS, the cells were incubated with species-specific Oregon Green-conjugated goat anti-rabbit IgG or Rhodamine Red-X-conjugated goat anti-mouse IgG (both from Molecular Probes, Eugene, OR). For double-label experiments using FtsZ-GFP, only the anti-tubulin and Rhodamine Red-X-conjugated goat anti-mouse antibodies were used together with the inherent green fluorescence of FtsZ-GFP. Intermediate filaments and actin were stained with monoclonal antibodies V9 (Sigma) and C4 (ICN Biomedicals, Costa Mesa, CA), respectively. In all cases, antibody specificity was tested by incubating coverslips with each primary antibody and the cross-species secondary antibody and making sure there was no signal by immunofluorescence. Potential cross-talk between the fluorescence channels on the microscope was tested by developing a strong signal with each of the primary and species-specific secondary antibody pairs, and viewing whether there was any signal in the non-appropriate channel.

Immunostained cells were viewed with a BX60 fluorescence microscope equipped with a 100W Mercury lamp and a 100× oil immersion fluorite objective (Olympus, Mellville, NY). A standard fluorescein isothiocyanate (FITC) filter set was used for GFP and Oregon Green, and a standard rhodamine filter was used for Rhodamine Red-X staining. The images were captured with a DEI-750 video camera (Optronics Engineering, Goleta, CA), digitized with an LG3 frame grabber (Scion, Frederick, MD), and viewed with the software program Photoshop 4.0 (Adobe Systems Inc., Mountain View, CA).

For time-lapse studies, transfected cells were grown on large round coverslips (22 mm diameter, Fisher Scientific, Pittsburgh, PA) and then inverted over a hanging drop slide (Fisher Scientific) containing medium with or without drug. To prevent drying out, the coverslips were sealed with VALAP (equal parts vaseline, lanolin and paraffin).

In some experiments, cells were pre-extracted to remove soluble proteins prior to fixation and immunofluorescence. To preserve microtubules during extraction, cells on coverslips were rinsed in PBS and then incubated on ice for 1 minute in microtubule stabilizing buffer (MTS) (10 mM Tris HCl, pH 6.8, 1 mM MgCl₂, 2 mM EGTA and 0.5% NP-40) containing 4 µg/ml paclitaxel (Sigma). FtsZ dots and filaments were preserved by a similar procedure using FtsZ stabilizing buffer (50 mM Tris HCl, pH 7.5, 1 mM MgCl₂, 1 mM PMSF, 0.5% NP-40, and 0.1 M potassium acetate) containing different concentrations of CaCl₂.

Pulse-chase studies

To determine the relative synthesis of FtsZ in CHO cells, clone 25.1

was grown overnight (20 hours) in medium with and without tetracycline and was then labeled for 1 hour by incubating the cells in MEM lacking methionine (Gibco) but containing 50 $\mu\text{Ci/ml}$ trans ^{35}S label (mixture of ^{35}S methionine and ^{35}S cysteine, >1000 Ci/mmol; ICN). For steady state labeling, the cells were similarly induced overnight without tetracycline and then incubated for 24 hours in 20 $\mu\text{Ci/ml}$ trans ^{35}S in normal medium. Lysis was carried out by scraping the cells in MTS buffer containing 0.14 M NaCl. Insoluble material was removed by centrifugation at 12,000 g for 5 minutes. Previous studies have shown that this procedure recovers >95% of the tubulin (Minotti et al., 1991) and virtually all of the FtsZ (X.-C. Yu, W. Margolin and F. Cabral, unpublished experiments). Proteins in the supernatant were precipitated by the addition of cold (4°C) acetone: NH_4OH (20:1) followed by centrifugation at 12,000 g for 5 minutes. Pellets were redissolved in isoelectric focusing dissociation buffer (8 M urea, 5 mM Tris HCl, pH 6.8, 1% Triton X-100, 1% 2-mercaptoethanol), and the proteins were resolved by two-dimensional gel electrophoresis as previously described (Cabral and Schatz, 1979; Sawada and Cabral, 1989). The relative amount of radioactivity incorporated into each of the proteins was measured from a digitized image of the gel acquired in a Storm phosphorimager using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Final images were obtained by exposing the dried gel to X-ray film (XAR-5, Eastman Kodak, Rochester, NY).

RESULTS

FtsZ accumulates to a similar level as endogenous β -tubulin

To circumvent the possibility that expression of FtsZ might prove toxic to mammalian cells, the gene was placed under the control of a tetracycline-regulated transactivator (Gossen and Bujard, 1992), and the transfected cells were maintained in tetracycline to prevent expression until the time of analysis. 24 stably transfected CHO cell lines were selected at random and screened for FtsZ production by immunofluorescence with affinity-purified anti-FtsZ antibody. Ten cell lines were positive by this screen. To obtain an estimate for the amount of FtsZ that was being produced, clone 25.1, a stably transfected cell line that appeared to exhibit the highest expression by immunofluorescence, was labeled with ^{35}S methionine for 60 minutes to measure protein synthesis, or was labeled overnight (20 hours) to measure steady state protein accumulation. Cell extracts were examined by two-dimensional gel electrophoresis and autoradiograms are shown in Fig. 1. The results indicated that little or no detectable FtsZ was produced by cells that were cultured in the presence of tetracycline (Fig. 1A). However, cells incubated overnight (20 hours) in medium without tetracycline before labeling, displayed a new spot with the predicted molecular mass and isoelectric point of FtsZ (arrow, Fig. 1B). The intensity of this new spot was approximately 3.5-fold higher than the intensity of β -tubulin, a protein that makes up approximately 1-2% of total cellular protein in mammalian cells (Hiller and Weber, 1978). Because FtsZ and mammalian β -tubulin have 16 and 17 methionines, respectively, the intensities of the spots should reflect their relative synthesis. When the stability of the FtsZ was assessed by chasing for 4 hours without radiolabel (Fig. 1C), or labeling the cells to steady state (Fig. 1D), the relative intensity of the FtsZ spot decreased about threefold but remained similar to that of tubulin, one of the most stable proteins in the cell (Boggs and Cabral, 1987; Spiegelman et al., 1977).

We conclude that FtsZ is not rapidly degraded in the transfected cells and that it can accumulate to a level that is comparable to endogenous tubulin. It has been reported that *E. coli* contains about 15,000 molecules of FtsZ per cell at a concentration of approximately 10 μM (Lu et al., 1998) and that tubulin is present at a concentration of 20 μM in mammalian cells (Hiller and Weber, 1978). Based on these numbers, we estimate that the transfected CHO cells have a similar concentration of FtsZ to *E. coli* cells.

FtsZ forms an array of dots that is independent of the mammalian cytoskeleton

Seven of the cell lines expressing FtsZ exhibited light diffuse fluorescence, but an additional three cell lines with higher production of FtsZ exhibited a striking pattern of intense cytoplasmic dots and nuclear dots and filaments (Fig. 2A). These dots were fairly uniform in size and appeared to be evenly spaced, an average of $2.5 \pm 0.4 \mu\text{m}$ apart. The percentage of cells with this pattern was in the range of 10-40% among the three different cell lines, and repeated attempts to clone out a subline with a higher percentage of positive cells were unsuccessful. Furthermore, the percentage of cells displaying dots did not diminish with time in culture, leading to the conclusion that this percentage is a characteristic feature of each cell line. Based on previous experience with transfected cell lines in which expression was found to be heterogeneous on a cell-to-cell basis (Barlow et al., 1994; Gonzalez-Garay and Cabral, 1995, 1996), we interpret these results to indicate that a critical amount of FtsZ is needed to form the dots, and that only a fraction of the cells in each population reaches the critical concentration. Expression of FtsZ was not toxic,

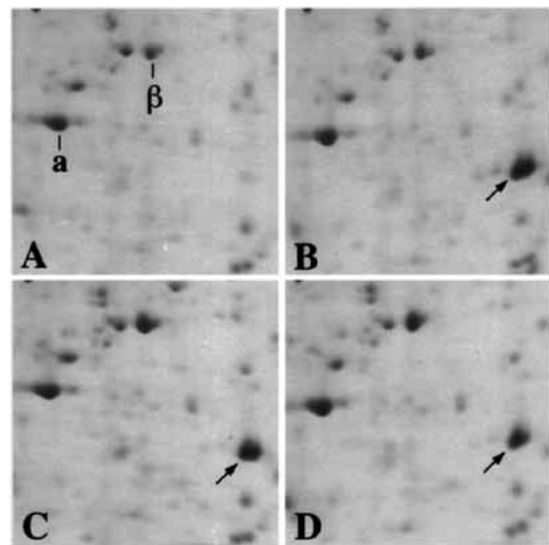


Fig. 1. Two-dimensional gels of FtsZ-producing cells. FtsZ clone 25.1 cells were kept in tetracycline (A) or induced for 20 hours in the absence of tetracycline (B-D) and were then labeled for 1 hour in ^{35}S methionine and lysed immediately (A,B) or chased with non-labeled medium for 4 hours before lysis (C). The cells in (D) were labeled continuously for 20 hours to obtain an estimate of steady state accumulation. Proteins in the cell lysates were resolved by two-dimensional gel electrophoresis and autoradiograms of the dried gels are shown. The basic end of the isoelectric focusing gel is to the left. a, actin; β , β -tubulin; arrows, FtsZ.

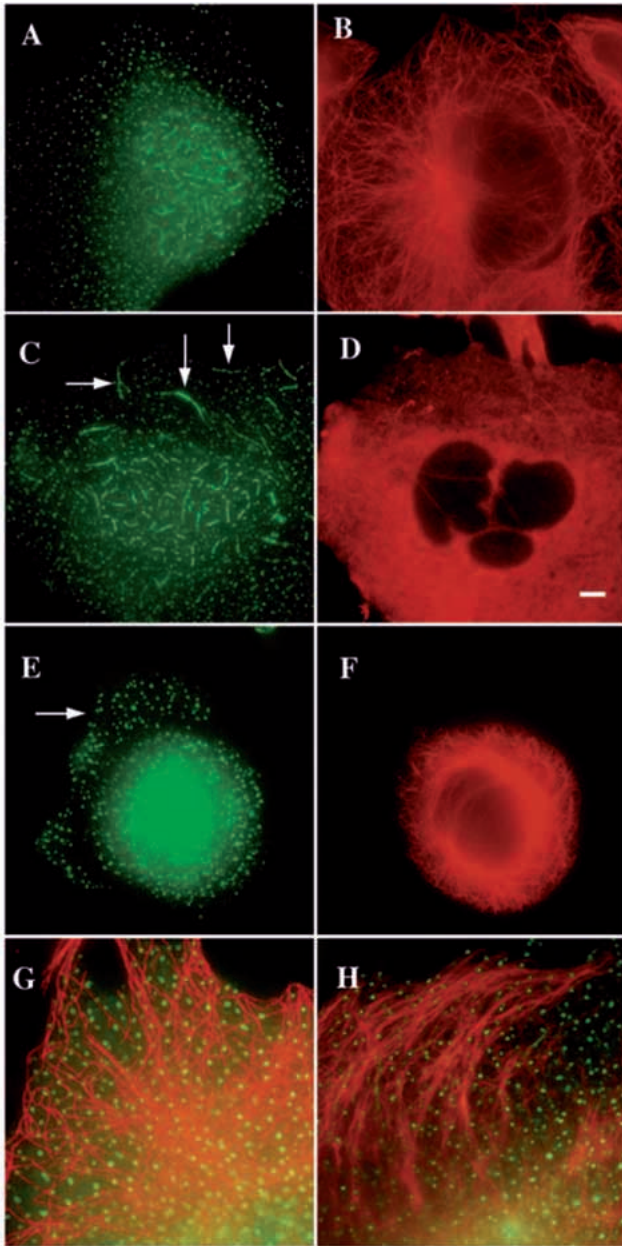


Fig. 2. FtsZ and the mammalian cytoskeleton. FtsZ clone 25.1 cells were induced for 20 hours in the absence of tetracycline, fixed and double stained for FtsZ (A,C,E) and α -tubulin (B,D,F). (G,H) Image overlays of FtsZ (green) and microtubules (G, red) or intermediate filaments (H, red). Cells were untreated (A,B), treated for 1 hour with 1 μ g/ml colcemid following induction (C,D), or treated for 20 hours during induction with 1 μ g/ml paclitaxel (E,F). Arrows in C indicate short FtsZ filaments seen in a subset of colcemid-treated cells. Arrow in E indicates the persistence of FtsZ dots in a section of the cytoplasm that is devoid of microtubules. Note that paclitaxel treatment has caused the microtubules to bundle around the nucleus. Bar, 5 μ m (A-F); 3 μ m (G,H).

because all cells grew equally well in the presence or absence of tetracycline. Cytoplasmic and nuclear dots were also observed by direct *in vivo* fluorescence when we introduced FtsZ-GFP into CHO cells using the identical plasmid system (see below). This demonstrates that the dots are not an artifact of fixation or the immunofluorescence staining procedure.

Because the three-dimensional structures of FtsZ and tubulin are very similar (Löwe and Amos, 1998; Nogales et al., 1998) and FtsZ polymerizes into protofilaments *in vitro* that are strikingly similar to tubulin protofilaments (Erickson et al., 1996), we examined the possibility that the cytoplasmic FtsZ dots resulting from high levels of FtsZ production might have a detectable effect on the assembly or distribution of microtubules. However, we have never noticed any disturbance in the microtubule network in cells that express FtsZ (e.g. Fig. 2B). We also noticed that CHO cells could produce FtsZ dots in all phases of the cell cycle including mitosis, and that FtsZ production did not appear to arrest the CHO cells at any point in the cell cycle or affect cell division or growth. The failure of FtsZ to disrupt microtubule structure or function suggests that despite similarities in their tertiary structures and assembly properties, FtsZ and tubulin do not coassemble under normal conditions.

We next explored the possibility that the localization of FtsZ might be guided by some type of interaction with microtubules. In double-label immunofluorescence experiments, FtsZ dots sometimes appeared to be associated with microtubules (Fig. 2G), but there was a lack of complete colocalization. To test more directly for the possibility of FtsZ/microtubule interactions, we treated clone 25.1 with 1 μ g/ml colcemid for 1 hour in an attempt to perturb the appearance or localization of the dots. This treatment efficiently depolymerized all the microtubules (Fig. 2D), but had little effect on the structure or distribution of FtsZ dots in most of the cells. However, in a few of the cells (approx. 5%), some short FtsZ filaments could be seen (arrows, Fig. 2C). Although short filaments were also occasionally seen in untreated controls, their frequency increased following colcemid treatment. Similar results were obtained with higher concentrations of the drug, prolonged treatment, or preincubation with colcemid prior to the induction of FtsZ (data not shown). Cells were also treated overnight with paclitaxel, a drug that caused microtubules to form bundles surrounding the nucleus (Fig. 2F). Despite the obvious change in the microtubule network, FtsZ dots remained normally distributed in the cytoplasm and persisted in peripheral regions of the cell that were lacking microtubules (arrow, Fig. 2E). We conclude that FtsZ dots form and distribute in the cytoplasm independently of the microtubule cytoskeleton. This conclusion is consistent with the presence of FtsZ dots and filaments in the nucleus, which is devoid of microtubules.

Finally, we tested the possible influence of other cytoskeletal elements in FtsZ dot formation and localization. Double-label immunofluorescence using antibodies to FtsZ and vimentin failed to show any association of the dots with intermediate filaments (Fig. 2H). In addition, when CHO cells synthesizing FtsZ were treated with cytochalasin B to disrupt the actin cytoskeleton, a clear change in the morphology of the cells was observed, but no change in the appearance of the dots could be seen (data not shown). We conclude from these studies that FtsZ dots form independently of the major cytoskeletal structures.

FtsZ dots formed *in vivo* are stable to extraction with Ca^{2+}

In vitro studies have demonstrated that millimolar levels of Ca^{2+} stimulate assembly of large FtsZ filaments (Yu and Margolin, 1997). To determine whether Ca^{2+} influences the

stability of FtsZ in mammalian cells, clone 25.1 cells were extracted in a non-ionic detergent-containing buffer with or without Ca^{2+} prior to fixation. As shown in Fig. 3B, cells extracted with 0.1 mM calcium lost all cytoplasmic, but not nuclear, FtsZ fluorescence. On the other hand, extraction with buffers containing more than 1 mM Ca^{2+} allowed retention of the cytoplasmic dots and reduced the background of diffuse FtsZ staining (Fig. 3C,D). Thus, the dots share at least one property (Ca^{2+} stability) that has been observed with FtsZ filaments in vitro, suggesting that they may also represent assembled FtsZ.

Rapid induction of FtsZ polymerization into filaments by vinblastine in vivo

As mentioned earlier, colcemid treatment caused FtsZ filaments, which were occasionally seen in the cytoplasm, to appear more frequently. We therefore investigated whether other anti-tubulin drugs might act as strong inducers of FtsZ filaments. Maytansine, podophyllotoxin, colchicine and nocodazole were similar to colcemid in having little or no effect on FtsZ dot formation or distribution, and did not induce appreciable FtsZ filaments (data not shown). In contrast, vinblastine rapidly and dramatically stimulated formation of an FtsZ filament network in the cytoplasm of virtually all cells expressing FtsZ.

Time-course experiments demonstrated that FtsZ filament formation is largely complete within 15 minutes of adding 1 $\mu\text{g/ml}$ vinblastine (Fig. 4A-C). However, some dots remain in a few cells even upon prolonged treatment. The filaments revert

to dots upon removal of the drug, but this is a slower process than their formation and requires approximately 1 hour to go to completion (Fig. 4D-F). By examining a large number of intermediate states in the process, we were left with the impression that filaments form between the dots which may get smaller as filaments form, but probably do not disappear. This was especially evident in the reversal experiment, when dots were again seen at regular intervals along the depolymerizing filaments (arrows, Fig. 4E, inset). We conclude that the dots are not simply aggregated protein, but may act as active nucleating centers for the FtsZ filaments, which then connect adjacent dots to give the appearance of long continuous filaments. Interestingly, we found that vinblastine had little or no effect on FtsZ dots and filaments in the nucleus. This observation, along with the inability of vinblastine to inhibit *E. coli* cell division (Casaregola et al., 1991) or promote FtsZ assembly in vitro (data not shown) suggests that vinblastine does not act by binding directly to FtsZ.

Time course of FtsZ-GFP polymerization in live CHO cells

An FtsZ-GFP fusion has been useful for monitoring the dynamics of FtsZ assembly in live *E. coli* cells and in vitro (Sun and Margolin, 1998; Yu and Margolin, 1997). To gain further insight into the formation of FtsZ filaments, we used a tetracycline-regulated FtsZ-GFP fusion to view the process in single CHO cells in real time. As with cells containing FtsZ, vinblastine treatment of cells producing FtsZ-GFP resulted in conversion of the cytoplasmic dots into filaments. The time course (Fig. 5) revealed that filaments first appeared in random and multiple areas of the cytoplasm as outgrowths from the FtsZ dots (arrows, 5 and 11 minute panels). With time, these filaments grew longer, appeared to connect several dots, and eventually formed a complex filamentous network (19-47 minute panels). In the final stages of filament formation, most dots could no longer be seen except for those in the nucleus. This series of events is very similar to the Ca^{2+} -mediated FtsZ polymerization in vitro previously reported (Yu and Margolin, 1997).

Tubulin filaments depend upon and coalign with FtsZ filaments after vinblastine treatment

To investigate the relationship between microtubule depolymerization and FtsZ filament formation, we determined the vinblastine dose-response for both processes. Concentrations of 0.01 $\mu\text{g/ml}$ and lower had no obvious effect on FtsZ dots or microtubules (Fig. 6A,B and data not shown). At 0.03 $\mu\text{g/ml}$, vinblastine greatly reduced or eliminated microtubules in many cells and induced the formation of cytoplasmic FtsZ filaments in approximately 5% of the cells (Fig. 6C,D). When the concentration was increased to 0.1-1 $\mu\text{g/ml}$, virtually all of the FtsZ-expressing cells exhibited at least some FtsZ filaments in the cytoplasm (Fig. 6E,G).

Surprisingly, at 0.1 $\mu\text{g/ml}$ of vinblastine, tubulin was organized into filaments that appeared to be largely coincident with FtsZ staining (Fig. 6E,F); but cells without significant FtsZ production had only diffuse tubulin staining (upper two cells, Fig. 6F). The results were even more dramatic at 1.0 $\mu\text{g/ml}$ vinblastine, which caused tubulin to form paracrystals in the FtsZ negative cells (arrows, Fig. 6H) but failed to prevent tubulin from forming filaments with FtsZ (Fig. 6G,H).

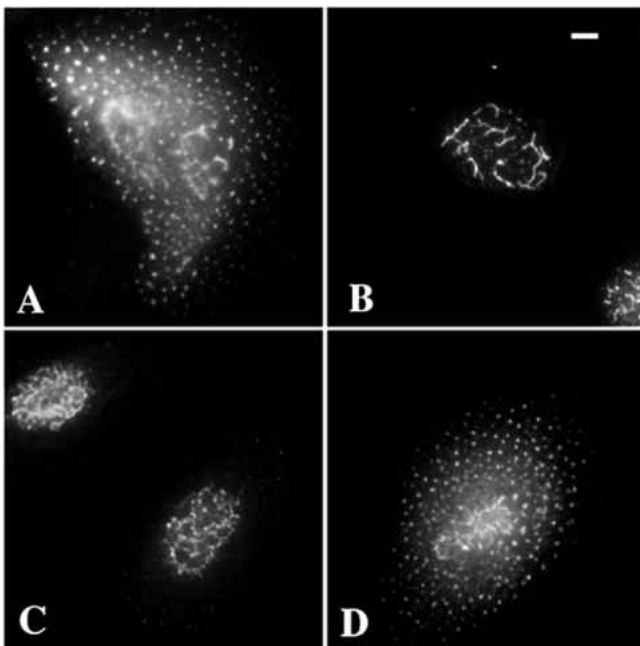


Fig. 3. Effect of Ca^{2+} on extraction of FtsZ. FtsZ clone 25.1 cells were induced for 20 hours without tetracycline and directly fixed (A) or extracted in FtsZ stabilizing buffer with 0.1 mM Ca^{2+} (B) 1 mM Ca^{2+} (C) or 10 mM Ca^{2+} (D) prior to fixation. The cells were then stained with an antibody to FtsZ. Note that dots are absent in the 0.1 mM Ca^{2+} extraction (B), weakly present in the 1 mM Ca^{2+} extraction (C) and well preserved in the 10 mM Ca^{2+} extraction (D). Bar, 5 μm .

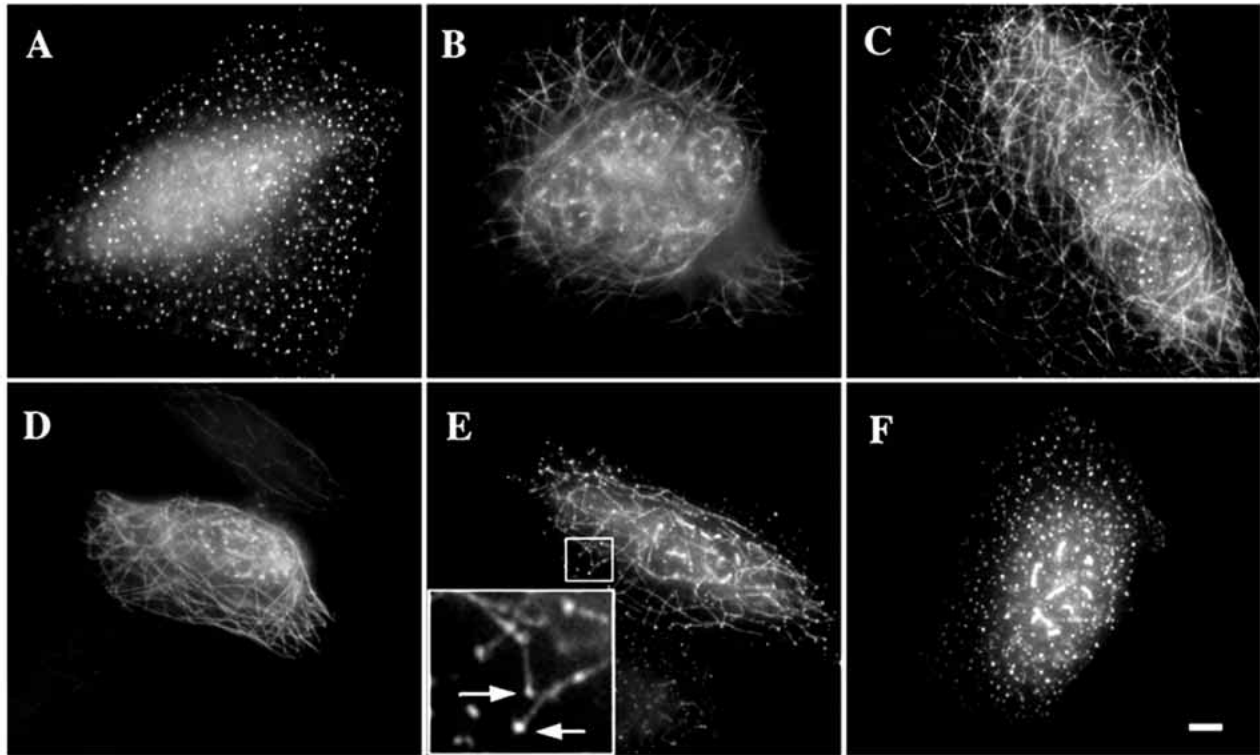


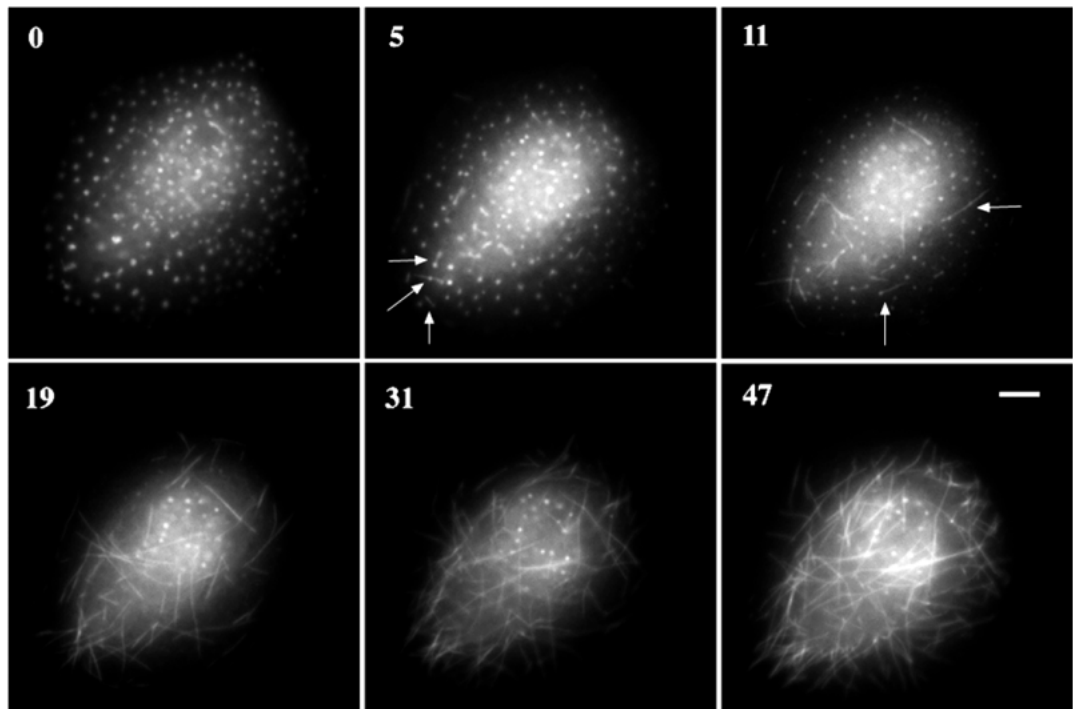
Fig. 4. FtsZ filament formation induced by vinblastine and its reversal. FtsZ clone 25.1 cells were induced for 20 hours without tetracycline and directly fixed (A) or treated with 1 $\mu\text{g/ml}$ vinblastine for 15 minutes (B) or 60 minutes (C-F). Cells were then fixed (B and C) or washed with $\alpha\text{-MEM}$ and incubated in $\alpha\text{-MEM}$ for 0 minutes (D), 30 minutes (E) or 60 minutes (F) prior to fixation. The cells were then stained with an antibody to FtsZ. Note the reappearance of dots along the depolymerizing filaments (inset arrows, E). Also note the persistence of dots in the nuclei of vinblastine treated cells (B-D). Bar, 5 μm .

Vincristine, a drug related to vinblastine, produced similar effects (data not shown).

Control experiments to test antibody specificity and

crossstalk between the two fluorescent channels ruled out the possibility that the apparent coalignment of FtsZ and tubulin in vinblastine-treated cells might be due to a staining artifact

Fig. 5. Time course of FtsZ-GFP filament formation in vinblastine-treated living cells. FtsZ-GFP clone 12.5 cells were induced 20 hours in medium without tetracycline followed by the addition of 1 $\mu\text{g/ml}$ vinblastine. FtsZ-GFP localization is shown in the same living cell at various times (minutes are shown as numbers in the upper left corner) after addition of the drug. Note at early time points that filaments appear to grow from the dots at random locations in the cytoplasm (arrows, 5 and 11 minute panels). Also note the retention of dots in the nucleus during filament formation in the cytoplasm. Bar, 5 μm .



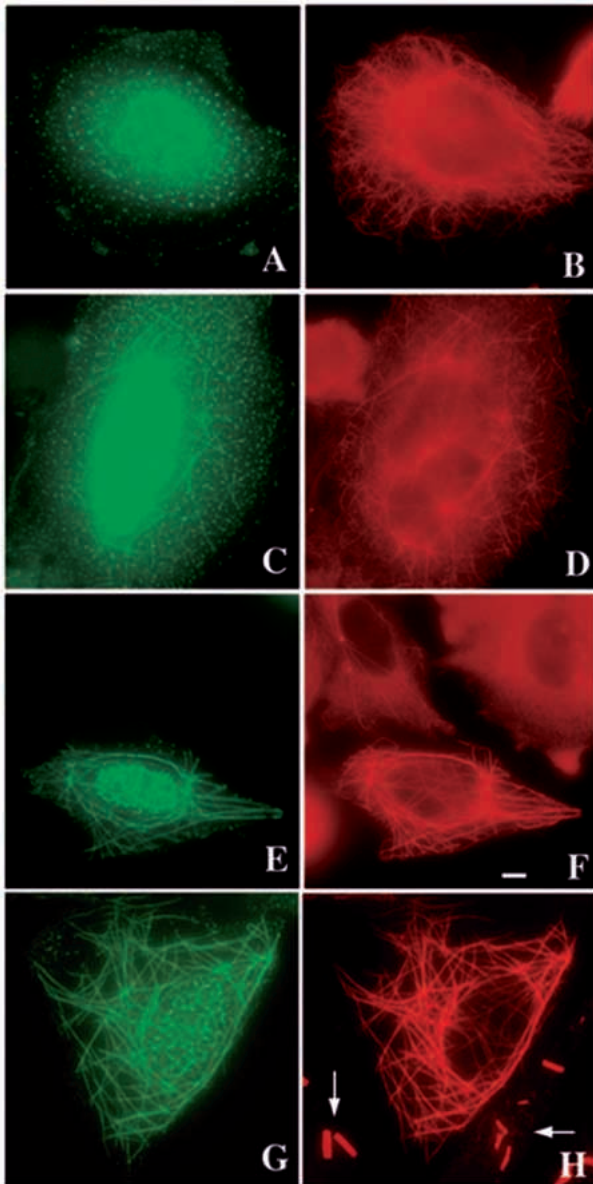


Fig. 6. Dose response to vinblastine. Clone 25.1 cells were induced for 20 hours without tetracycline and then treated for 1 hour with 0 (A,B), 0.03 (C,D), 0.1 (E,F) or 1 $\mu\text{g/ml}$ vinblastine (G,H). The cells were then fixed and double stained for FtsZ (A,C,E,G) and α -tubulin (B,D,F,H). Arrows in H indicate tubulin paracrystal formation in cells that are not expressing FtsZ. Bar, 5 μm .

(see Materials and methods). Furthermore, FtsZ nuclear staining was never observed in the tubulin channel and tubulin paracrystals were never observed in the FtsZ channel (e.g. Fig. 6G,H). We conclude from these experiments that microtubule disassembly and detectable FtsZ filament formation occur at approximately the same concentration of vinblastine, and that the presence of FtsZ allows tubulin polymers to form in concentrations of the drug that should cause their disassembly or precipitation into paracrystals.

To test whether pre-existing microtubules are required for FtsZ filament formation, we pretreated clone 25.1 cells with vinblastine before induction of FtsZ expression to remove all

microtubules hours before significant amounts of FtsZ could accumulate. Despite the vinblastine pretreatment, FtsZ filaments still formed and had coaligned tubulin polymers as before (data not shown). It thus appears that preexisting microtubules are not required for the formation of FtsZ filaments in vinblastine-treated cells, and that the tubulin polymers found coaligned with FtsZ are newly formed structures rather than rearranged microtubules.

Vinblastine effects on FtsZ assembly are mediated by tubulin

The dose-response for vinblastine induction of FtsZ filaments, the drug's inability to influence FtsZ assembly in vitro or in the nucleus of transfected cells, and the lack of vinblastine toxicity in bacterial cells (Casaregola et al., 1991; and data not shown) all argue that the effects of vinblastine require the participation of tubulin. To further demonstrate tubulin involvement, we investigated whether other microtubule binding drugs could inhibit the effects of vinblastine. Colcemid, a drug that binds to tubulin at a site distinct from vinblastine, had little effect on the ability of vinblastine to induce FtsZ polymers regardless of whether it was added prior to, along with, or following addition of vinblastine (Fig. 7A,B). In contrast, maytansine, a drug that binds to the vinblastine binding site on tubulin but has no ability of its own to induce FtsZ filaments (data not shown), prevented the formation of FtsZ filaments when added 1 hour before or at the same time as vinblastine (Fig. 7C,D). Furthermore, maytansine was able to reverse filament formation in cells that had been pretreated with vinblastine. For example, when cells were treated with vinblastine for 1 hour and maytansine was then added for an additional hour (without removing the vinblastine), 30% of cells totally reverted to FtsZ dots (not shown) while the remaining 70% of the cells had a greatly reduced number of filaments (Fig. 7E,F). The ability of maytansine to prevent or reverse FtsZ polymer formation provides further evidence that vinblastine's effects on FtsZ assembly are mediated through binding to tubulin.

FtsZ and tubulin segregate into separate polymers following vinblastine treatment

The coalignment of FtsZ and tubulin filaments in vinblastine-treated cells could potentially be explained by lateral association of independent filaments or by copolymerization of FtsZ with tubulin. To discriminate between these two possibilities, cells expressing FtsZ were treated with vinblastine and were then extracted with microtubule stabilizing buffer prior to fixation and immunofluorescence staining. As we had previously observed with FtsZ dots (data not shown), this procedure resulted in the loss of FtsZ filaments, but the tubulin polymers remained (Fig. 8C,D; compare to unextracted cells in Fig. 8A,B). The disappearance of FtsZ but not tubulin polymers indicates that the two proteins are not copolymerized. Interestingly, extraction with FtsZ stabilizing buffer that contained 10 mM Ca^{2+} , which normally causes the disappearance of microtubules (data not shown), had little effect on the vinblastine-dependent tubulin polymers. In this case, both FtsZ and tubulin polymers remained after extraction (Fig. 8E,F). This result suggests either that these tubulin polymers are themselves Ca^{2+} -resistant, or that under

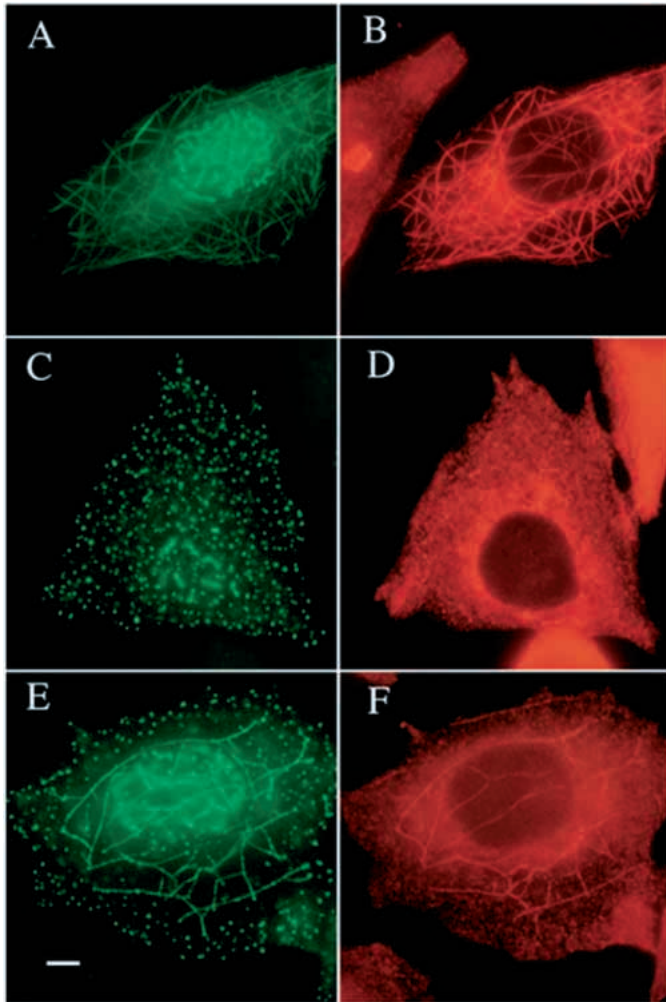


Fig. 7. Maytansine induced reversal of FtsZ filaments. FtsZ clone 25.1 cells were induced for 20 hours without tetracycline. The cells were then treated with a mixture of 1 $\mu\text{g/ml}$ vinblastine plus either 1 $\mu\text{g/ml}$ colcemid (A,B) or 1 $\mu\text{g/ml}$ maytansine (C,D) for 1 hour and were then fixed in methanol. The cells in E and F were pretreated for 1 hour with 1 $\mu\text{g/ml}$ vinblastine alone, and then 1 $\mu\text{g/ml}$ maytansine was added for an additional hour before fixation. Double immunofluorescence was carried out with antibodies to FtsZ (A,C,E) and tubulin (B,D,F). Note that maytansine, but not colcemid, prevented the formation of FtsZ filaments when added together with vinblastine (C,D) and was able to significantly reverse preformed filaments even in the continued presence of vinblastine (E,F). Bar, 5 μm .

these buffer conditions FtsZ can protect the tubulin polymers from Ca^{2+} -induced depolymerization.

DISCUSSION

Polymers of FtsZ and tubulin perform essential cytoskeletal functions in prokaryotes and eukaryotes, respectively. Their striking structural similarity and high degree of conservation prompted us to investigate the effects of expressing FtsZ in a eukaryote. The converse experiment, expression of functional tubulin in bacteria, is problematic because tubulin folding requires a complex mixture of chaperones, a chaperonin complex, and at least five additional cofactors (Lewis et al.,

1997; Liang and MacRae, 1997). The prospect of expressing FtsZ in mammalian cells raises several interesting questions. For example, will FtsZ form a functional protein in a non-bacterial cell or does it require a prokaryotic-specific chaperone for folding? Will FtsZ localize to specific cellular regions, thus providing a hint as to how FtsZ localizes to the division plane in bacteria? Can FtsZ coassemble with microtubules and, if so, will it interfere with microtubule function?

We have succeeded in answering these questions. Assembly-competent FtsZ was produced in CHO cells at levels that were comparable to endogenous β -tubulin and to the levels that are normally found in bacteria. However, this amount of FtsZ had no detectable effect on the microtubule network or cell growth. We conclude that under normal conditions FtsZ does not interact with tubulin or any other known cytoskeletal component enough to impair function. At lower protein concentrations, FtsZ appeared to be soluble, based on the observed diffuse fluorescence pattern. However, at higher concentrations, FtsZ formed dots that were uniformly distributed throughout the cytoplasm in a striking punctate pattern. In addition, FtsZ also assembled into distributed dots and filaments within the nucleus.

The cytoplasmic distribution pattern is reminiscent of the behavior of overproduced FtsZ-GFP in *E. coli*. Under these conditions, the fusion protein forms a linear array of dots at potential division sites in filamentous bacterial cells (Ma et al., 1996). The distribution of FtsZ dots in CHO cells appears to be a three-dimensional manifestation of this one-dimensional bacterial phenotype, and is remarkable considering it is a foreign protein. Moreover, the spacing between FtsZ dots in CHO cells ($2.5 \pm 0.4 \mu\text{m}$) is in the same range as the 2–3 μm spacing between FtsZ rings or dots in *E. coli* cells. The mechanism by which FtsZ dots are spaced is unknown, but we speculate that FtsZ may in some way be able to specify its own distribution. Considering this possibility, it is interesting to note that FtsZ rings can form at the midpoint of *E. coli* cells of various sizes which are devoid of any obvious spatial cues such as chromosomes (Sun et al., 1998).

Another possibility is that the FtsZ dots are attached to a cellular factor or organelle that is itself arranged in a periodic manner. If true, it would be important to discover its identity as it might share some characteristics with a putative receptor at the bacterial division site. However, we think this idea is less likely for several reasons. First, the dots seem to be strictly dependent on expression level and are almost always above a minimum size, at least as judged by fluorescence. Thus, it makes more sense for the spatial distribution of FtsZ to be coupled to its assembly. Second, disruption of microtubules or the actin cytoskeleton has no effect on the distribution of FtsZ dots. Third, the FtsZ dots do not seem to colocalize with microtubules or with intermediate filaments. Fourth, attempts to immunoprecipitate specific cellular proteins interacting with FtsZ-GFP using an antibody to GFP were unsuccessful (X.-c. Yu, W. Margolin and F. Cabral, unpublished experiments). Finally, a similar distribution of dots occurs in the nucleus, which should be largely devoid of common structures with the cytoplasm.

Subsequent investigation into the formation and dynamics of the FtsZ dots suggested that they are reservoirs for assembly of large polymers. The ability of the dots to generate polymers is

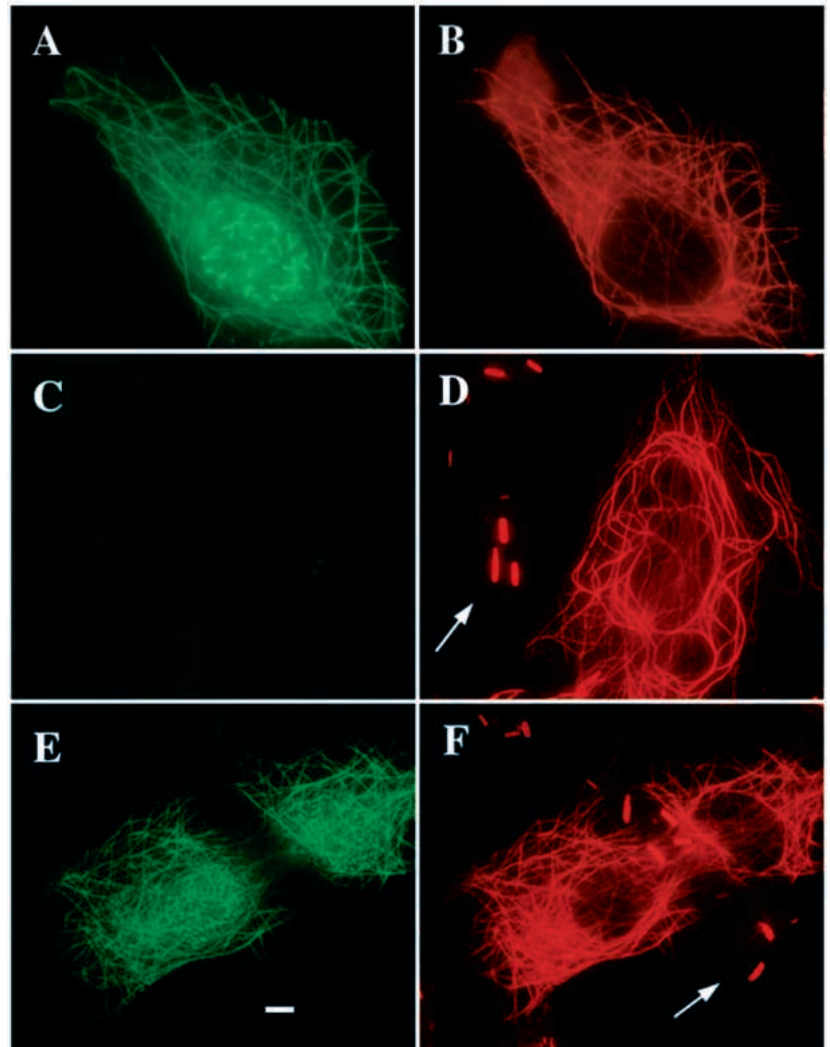


Fig. 8. Extraction of vinblastine-induced FtsZ filaments. FtsZ clone 25.1 cells were induced for 20 hours without tetracycline, treated for 1 hour with 1 $\mu\text{g/ml}$ vinblastine, and then fixed (A,B). Cells in the remaining panels were first extracted with microtubule stabilizing buffer (C,D) or with FtsZ stabilizing buffer containing 10 mM Ca^{2+} (E,F) before fixation. The extracted cells were double stained for FtsZ (A,C,E) and α -tubulin (B,D,F). Arrows indicate cells that were not expressing FtsZ. Note in F that a cell containing tubulin paracrystals, which does not express FtsZ, overlaps two cells that express FtsZ and form filaments. Bar, 5 μm .

significant because (1) it demonstrates that the dots are active nucleation centers rather than repositories of inactive protein, and (2) it further demonstrates that FtsZ assembly can occur in the absence of any bacterial cofactors. In the time course experiments with FtsZ-GFP in live CHO cells, FtsZ-GFP polymers were seen to emanate from the dots and connect with other dots, which became less prominent with time. This behavior, along with the stabilization of the dots by Ca^{2+} ions, is remarkably similar to the properties of FtsZ polymers in vitro (Yu and Margolin, 1997). The rapid formation of polymers from the dots, as well as our demonstration that dots can be regenerated from polymers, indicated that the whole process is dynamic. It is notable that in large, spherical *E. coli* mutant cells, FtsZ ring assembly appears to originate from a single nucleation center by first forming an arc (Addinall and Lutkenhaus, 1996), much as filaments appear to emanate from the dots in CHO cells.

In light of the necessity of a complex folding pathway for the formation of native tubulin (Lewis et al., 1997), our expression studies were important because they demonstrated that assembly competent FtsZ protein can be produced in CHO cells without the participation of any bacterial chaperone. This suggests either that (1) FtsZ folding does not require chaperones, or (2) eukaryotic chaperones can substitute for the

normally required bacterial homologs to assist FtsZ folding. It should be noted that we have successfully refolded purified FtsZ solubilized from bacterial inclusion bodies, suggesting that chaperones may facilitate, but may not be required, for folding in vivo (Yu and Margolin, 1997). This contrasts sharply with tubulin, for which there are few reports of refolding from similar types of inclusion bodies (Tian et al., 1996).

A major motivation for this project was to test for possible functional interactions between FtsZ and tubulin. One such interaction was indeed discovered: the widespread coalignment of FtsZ and tubulin polymers after vinblastine treatment and the apparent dependence of the tubulin polymers on the presence of FtsZ, as indicated by their absence in cells that failed to express the bacterial protein. Moreover, FtsZ and tubulin filaments were found to coalign even when cells were pretreated with vinblastine prior to induction of FtsZ synthesis. This suggests that FtsZ does not directly stabilize pre-existing microtubules, but instead may efficiently aid tubulin polymerization under these conditions. Consistent with this idea is the very different morphological appearance of the FtsZ polymer network compared to the normal microtubule network; it is evident that FtsZ polymers are following the tracks of FtsZ dots, not microtubules.

The colocalization of tubulin and FtsZ in vinblastine-treated cells suggested the possibility that the two structurally similar proteins might assemble into a copolymer. However, the ability to remove FtsZ, but not tubulin, from the coaligned polymers argues strongly against such an idea. Although we do not know whether the tubulin polymers that we see by immunofluorescence are normal microtubule structures, they do share some properties with microtubules. For example, they are stable to extraction in microtubule stabilizing buffer; and they contain α -tubulin, β -tubulin and MAP 4 (X.-c. Yu, W. Margolin and F. Cabral, unpublished experiments). On the other hand, they have a distinct organizational pattern and they are stable at concentrations of vinblastine and calcium that should cause them to depolymerize. How much of these latter features are due to inherent differences in structure versus their interaction with FtsZ is unknown. Furthermore, we cannot rule out the possibility that interaction between tubulin and FtsZ is mediated by other proteins. In this regard, however, it should be noted that immunoprecipitation experiments did not reveal any interactions of FtsZ with cellular proteins either in the presence or absence of vinblastine (X.-c. Yu, W. Margolin and F. Cabral, unpublished experiments), and that immunofluorescence experiments ruled out the potential involvement of intermediate filaments in the formation of FtsZ/tubulin polymers. Moreover, purified FtsZ and tubulin can form what appear to be coaligned polymers in vitro (X.-c. Yu and W. Margolin, unpublished observations), suggesting that other cellular proteins are not required for lateral association.

The mechanism by which vinblastine and vincristine induce the coaligned assembly of FtsZ and tubulin polymers is unknown. However, we favor the possibility that binding of these drugs induces a conformational change in tubulin that decreases its ability to self-assemble but promotes its ability to interact with FtsZ. The idea that vinblastine's effects are mediated through changes in tubulin is consistent with the observations that microtubule disassembly and detectable FtsZ filament formation occurred at roughly the same concentration of vinblastine, that the drug had no effect on the growth of bacteria or on the polymerization of FtsZ in vitro, and that the occasional FtsZ polymers seen in untreated or colcemid-treated cells did not have coaligned tubulin polymers (Yu and Margolin, 1998; X.-c. Yu, W. Margolin and F. Cabral, unpublished experiments). An additional clue comes from the fact that maytansine, which also binds to the vinblastine binding site on tubulin (Bhattacharyya and Wolff, 1977), fails to induce FtsZ polymer formation, but can effectively reverse the effects of vinblastine. Therefore, if vinblastine-mediated FtsZ polymerization is mediated via a conformational change in tubulin, that change is not replicated by maytansine. This is not surprising, because maytansine and vinblastine induce very different structural changes in tubulin; for example, maytansine is incapable of inducing the formation of tubulin spirals and paracrystals associated with high concentrations of vinblastine (Bensch and Malawista, 1969; Bhattacharyya and Wolff, 1977; Malawista and Sato, 1969; Sackett, 1995). Thus, drugs such as vinblastine and vincristine appear to be unique in their ability to induce a change in tubulin that results in the formation of novel tubulin polymers. It may be this unique change in tubulin that also allows it to interact with FtsZ.

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REFERENCES

- Addinall, S. G. and Lutkenhaus, J.** (1996). FtsZ-spirals and -arcs determine the shape of the invaginating septa in some mutants of *Escherichia coli*. *Mol. Microbiol.* **22**, 231-237.
- Barlow, S. B., Gonzalez-Garay, M. L., West, R. R., Olmsted, J. B. and Cabral, F.** (1994). Stable expression of heterologous microtubule associated proteins in Chinese hamster ovary cells: evidence for differing roles of MAPs in microtubule organization. *J. Cell Biol.* **126**, 1017-1029.
- Bensch, K. G. and Malawista, S. E.** (1969). Microtubular crystals in mammalian cells. *J. Cell Biol.* **40**, 95-106.
- Bhattacharyya, B. and Wolff, J.** (1977). Maytansine binding to the vinblastine sites of tubulin. *FEBS Lett.* **75**, 159-162.
- Bi, E. and Lutkenhaus, J.** (1991). FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**, 161-164.
- Boggs, B. and Cabral, F.** (1987). Mutations affecting assembly and stability of tubulin: evidence for a non-essential β -tubulin in CHO cells. *Mol. Cell. Biol.* **7**, 2700-2707.
- Bramhill, D.** (1997). Bacterial cell division. *Annu. Rev. Cell Dev. Biol.* **13**, 395-424.
- Cabral, F. and Schatz, G.** (1979). One- and two-dimensional electrophoretic analysis of mitochondrial membrane proteins. *Methods Enzymol.* **56**, 602-613.
- Casaregola, S., Chen, M., Bouquin, N., Norris, V., Jacq, A., Goldberg, M., Margaron, S., Tempete, M., McKenna, S., Sweetman, H., Bernard, S., McGurk, G., Seror, S. and Holland, I. B.** (1991). Analysis of a myosin-like protein and the role of calcium in the *E. coli* cell cycle. *Res. Microbiol.* **142**, 201-207.
- Cormack, B. P., Valdivia, R. H. and Falkow, S.** (1996). FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**, 33-38.
- de Boer, P., Crossley, R. and Rothfield, L.** (1992). The essential bacterial cell-division protein FtsZ is a GTPase. *Nature* **359**, 254-256.
- Erickson, H. P.** (1997). FtsZ, a tubulin homologue in prokaryote division. *Trends Cell Biol.* **7**, 362-367.
- Erickson, H. P., Taylor, D. W., Taylor, K. A. and Bramhill, D.** (1996). Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. *Proc. Natl. Acad. Sci. USA* **93**, 519-523.
- Faguy, D. M. and Doolittle, W. F.** (1998). Cytoskeletal proteins: the evolution of cell division. *Curr. Biol.* **8**, 338-341.
- Gonzalez-Garay, M. L. and Cabral, F.** (1995). Overexpression of an epitope-tagged β -tubulin in Chinese hamster ovary cells causes an increase in endogenous α -tubulin synthesis. *Cell Motil. Cytoskel.* **31**, 259-272.
- Gonzalez-Garay, M. L. and Cabral, F.** (1996). α -tubulin limits its own synthesis: evidence for a mechanism involving translational repression. *J. Cell Biol.* **135**, 1525-1534.
- Gonzalez-Garay, M. L., Chang, L., Blade, K., Menick, D. R. and Cabral, F.** (1999). A β -tubulin leucine cluster involved in microtubule assembly and paclitaxel resistance. *J. Biol. Chem.* (in press).
- Gossen, M. and Bujard, H.** (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* **89**, 5547-5551.
- Hiller, G. and Weber, K.** (1978). Radioimmunoassay for tubulin: a quantitative comparison of the tubulin content of different established tissue culture cells and tissues. *Cell* **14**, 795-804.
- Lewis, S. A., Tian, G. and Cowan, N. J.** (1997). The α - and β -tubulin folding pathways. *Trends Cell Biol.* **7**, 479-484.
- Liang, P. and MacRae, T. H.** (1997). Molecular chaperones and the cytoskeleton. *J. Cell Sci.* **110**, 1431-1440.
- Löwe, J. and Amos, L. A.** (1998). Crystal structure of the bacterial cell-division protein FtsZ. *Nature* **391**, 203.
- Lu, C., Stricker, J. and Erickson, H. P.** (1998). FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritima*—quantitation, GTP hydrolysis, and assembly. *Cell Motil. Cytoskel.* **40**, 71-86.
- Lutkenhaus, J. and Addinall, S. G.** (1997). Bacterial cell division and the Z ring. *Ann. Rev. Biochem.* **66**, 93-116.
- Ma, X., Ehrhardt, D. W. and Margolin, W.** (1996). Colocalization of cell division proteins FtsZ and FtsA to cytoskeletal structures in living

- Escherichia coli* cells by using green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **93**, 12998-13003.
- Malawista, S. E. and Sato, H.** (1969). Vinblastine produces uniaxial, birefringent crystals in starfish oocytes. *J. Cell Biol.* **42**, 596-599.
- Margolin, W.** (1998). A green light for the bacterial cytoskeleton. *Trends Microbiol.* **6**, 233-238.
- Margolin, W., Wang, R. and Kumar, M.** (1996). Isolation of an *ftsZ* homolog from the archaeobacterium *Halobacterium salinarium*: implications for the evolution of FtsZ and tubulin. *J. Bacteriol.* **178**, 1320-1327.
- Minotti, A. M., Barlow, S. B. and Cabral, F.** (1991). Resistance to antimetabolic drugs in Chinese hamster ovary cells correlates with changes in the level of polymerized tubulin. *J. Biol. Chem.* **266**, 3987-3994.
- Mukherjee, A., Dai, K. and Lutkenhaus, J.** (1993). *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc. Natl. Acad. Sci. USA* **90**, 1053-1057.
- Mukherjee, A. and Lutkenhaus, J.** (1998). Dynamic assembly of FtsZ regulated by GTP hydrolysis. *EMBO J.* **17**, 462-469.
- Nogales, E., Wolf, S. G. and Downing, K. H.** (1998). Structure of the alpha-beta tubulin dimer by electron crystallography. *Nature* **391**, 199-203.
- Osteryoung, K. W., Stokes, K. D., Rutherford, S. M., Percival, A. L. and Lee, W. Y.** (1998). Chloroplast division in higher plants requires members of two functionally divergent gene families with homology to bacterial *ftsZ*. *Plant Cell* **10**, 1991-2004.
- Raychaudhuri, D. and Park, J. T.** (1992). *Escherichia coli* cell-division gene *ftsZ* encodes a novel GTP-binding protein. *Nature* **359**, 251-254.
- Rothfield, L. I. and Justice, S. S.** (1997). Bacterial cell division: the cycle of the ring. *Cell* **88**, 581-584.
- Sackett, D. L.** (1995). Vinca site agents induce structural changes in tubulin different from and antagonistic to changes induced by colchicine site agents. *Biochemistry* **34**, 7010-7019.
- Sawada, T. and Cabral, F.** (1989). Expression and function of β -tubulin isotypes in Chinese hamster ovary cells. *J. Biol. Chem.* **264**, 3013-3020.
- Spiegelman, B. M., Penningroth, S. M. and Kirschner, M. W.** (1977). Turnover of tubulin and the N site GTP in Chinese hamster ovary cells. *Cell* **12**, 587-600.
- Strepp, R., Scholz, S., Kruse, S., Speth, V. and Reski, R.** (1998). Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. *Proc. Natl. Acad. Sci. USA* **95**, 4368-4373.
- Sun, Q. and Margolin, W.** (1998). FtsZ dynamics during the cell division cycle of live *Escherichia coli*. *J. Bacteriol.* **180**, 2050-2056.
- Sun, Q., Yu, X.-C. and Margolin, W.** (1998). Assembly of the FtsZ ring at the central division site in the absence of the chromosome. *Mol. Microbiol.* **29**, 491-504.
- Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C. and Cowan, N. J.** (1996). Pathway leading to correctly folded beta-tubulin. *Cell* **86**, 287-296.
- Wang, X. and Lutkenhaus, J.** (1996). FtsZ ring: the eubacterial division apparatus conserved in archaeobacteria. *Mol. Microbiol.* **21**, 313-320.
- Weisenberg, R. C.** (1972). Microtubule formation in vitro in solutions containing low calcium concentrations. *Science* **177**, 1104-1105.
- Yu, X.-C. and Margolin, W.** (1997). Ca²⁺-mediated GTP-dependent dynamic assembly of bacterial cell division protein FtsZ into asters and polymer networks in vitro. *EMBO J.* **16**, 5455-5463.
- Yu, X.-C. and Margolin, W.** (1998). Inhibition of assembly of bacterial cell division protein FtsZ by the hydrophobic dye bis-8-anilino-1-naphthalenesulfonate. *J. Biol. Chem.* **273**, 10216-10222.
- Yu, X.-C., Tran, A. H., Sun, Q. and Margolin, W.** (1998). Localization of cell division protein FtsZ to the *Escherichia coli* septum and identification of a potential N-terminal targeting domain. *J. Bacteriol.* **180**, 1296-1304.