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Polymerization of the bacterial cell division protein FtsZ

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Chapter 5

Substitution of a conserved aspartate allows cation-induced polymerization of FtsZ

Dirk-Jan Scheffers, Janny G. de Wit, Tanneke den Blaauwen and Arnold J.M. Driessen

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Summary

The prokaryotic tubulin homologue FtsZ polymerizes in vitro in a nucleotide dependent fashion. Here we report that replacement of the strictly conserved Asp212 residue of *Escherichia coli* FtsZ by a Cys or Asn, but not by a Glu residue results in FtsZ that polymerizes with divalent cations in the absence of added GTP. FtsZ D212C and D212N mutants co-purify with GTP as bound nucleotide, providing an explanation for the unusual phenotype. We conclude that D212 plays a critical role in the coordination of a metal ion and the nucleotide at the interface of two FtsZ monomers.

Introduction

The key prokaryotic cell division protein FtsZ forms a structural element known as the Z-ring at the site of cell division (for a recent review see ref. 92). FtsZ has been identified in all but four prokaryotic species studied to date (49) and is essential for division of chloroplasts and mitochondria in some eukaryotes (7, 111). In *Escherichia coli*, the Z-ring is critical for the localization of all other known protein components of the cell division machinery to the division site (92). The Z-ring is likely to consist of polymers similar to the FtsZ polymers that can be formed in vitro in a GTP dependent manner (19, 51, 101)

FtsZ is the prokaryotic homologue of tubulin (48, 106). Both proteins share the unique GTP-binding motif known as G-box (34). FtsZ displays a concentration dependent GTPase and polymerization activity suggesting that it acts through self-activation (81, 102, 128). A comparison of tubulin and FtsZ structures arranged in their polymerized forms indicated an important role for the T7-loop of FtsZ in modulation of the GTPase activity. This loop may interact with the γ-phosphate of the GTP bound to the nucleotide-binding site on another monomer (78, 107). This hypothesis is augmented by various mutations in the T7-loop that abolish or inhibit FtsZ GTPase activity and polymerization (31, 137, 145)(chapter 4).

The aspartate residue at position 212 of the T7-loop is conserved among all FtsZ amino acid sequences known to date. This residue is homologous to a glutamate in α-tubulin that modulates the hydrolysis of the GTP bound to β-tubulin, whereas a lysine at the similar position in β-tubulin prevents hydrolysis of the GTP bound to α-tubulin (106). The D212G mutation in *E. coli* FtsZ abolishes GTPase but not GTP-binding activity and renders the protein resistant to the SulA, an inhibitor of FtsZ polymerization (31, 99, 137). Here we report that FtsZ D212C and D212N mutants polymerize in the presence of divalent cations, without added GTP or GDP. The tightly bound nucleotide that copurifies with these mutants is GTP, in contrast to wild-type FtsZ or the D212E mutant that contain bound GDP. Our results point at a critical role for D212 in nucleotide hydrolysis and coordination of the cation at the nucleotide-binding site.

Results

Characteristics of the D212 mutants

Site-directed FtsZ D212N, D212E and D212C mutants were constructed to analyze the role of the T7-loop in GTP hydrolysis and polymerization (chapter 4). All D212 mutants displayed a markedly reduced GTP hydrolysis activity (Fig. 1). FtsZ D212E polymerized with GTP in the presence of calcium, albeit at an increased critical protein concentration for polymerization. FtsZ D212N and D212C, however, behaved different from wt FtsZ. First, a high basal level of light scattering precluded the spectroscopical analysis of the polymerization behavior of these mutants. Second, in the sedimentation assay, these mutants also appeared to polymerize with GDP (not shown). These unusual characteristics prompted us to characterize these D212 mutants in more detail.

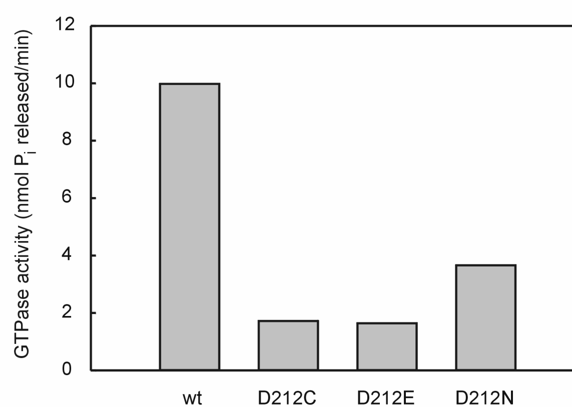

Figure 1. GTPase activity of wt FtsZ and FtsZ D212C, D212E and D212N mutant proteins. GTP hydrolysis was measured as described in the text.

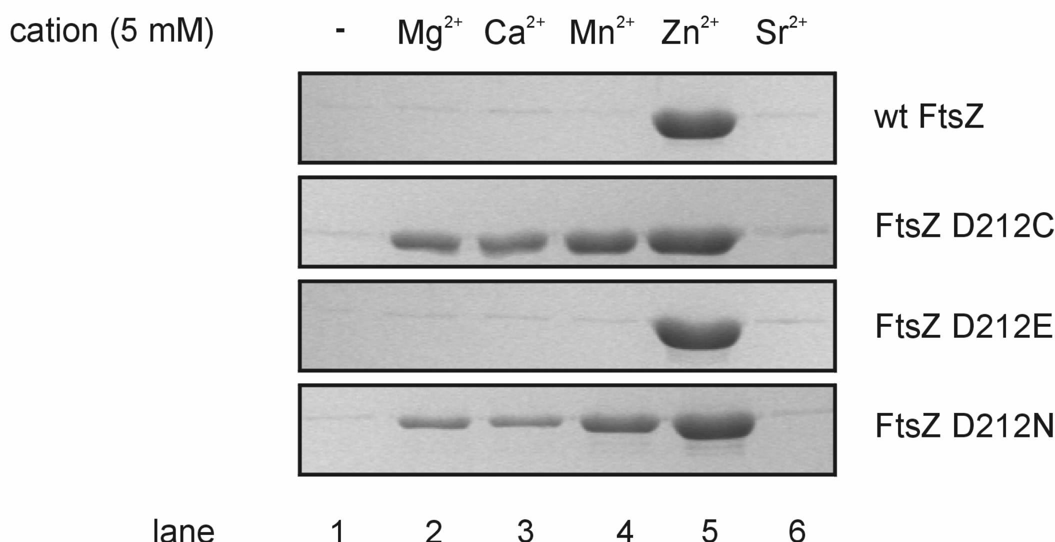

Figure 2. Sedimentation of wt FtsZ and FtsZ D212C, D212E and D212N mutant proteins in the presence of various cations. Protein was incubated for 5 min at 30°C in polymerization buffer with no additions (lane 1) or 5 mM $MgCl_2$ (lane 2); $CaCl_2$ (lane 3); $MnCl_2$ (lane 4); $ZnCl_2$ (lane 5) or $SrCl_2$ (lane 6). Sedimentation and analysis of the protein pellets was performed as described in the materials and methods section.

Removal of the negative charge at position 212 allows cation-induced polymerization

Since FtsZ D212N and D212C sedimented irrespective of the nature of the nucleotide, the polymerization solution was analyzed for another factor that induces sedimentation. Omission of $MgCl_2$ completely abolished sedimentation (Fig 2). In the absence of added nucleotide, addition of 5 mM of $MgCl_2$, $CaCl_2$, $MnCl_2$, $CoCl_2$, and $NiCl_2$ led to the sedimentation of substantial amounts of FtsZ D212N and D212C (Fig. 2, Table 1), whereas wt FtsZ and FtsZ D212E did not sediment. Since $SrCl_2$ or $BaCl_2$ were unable to induce sedimentation (Fig. 2), the size exclusion limit for the (crystal) cation radius is ~ 1.1 Å (Table 1). With 5 mM $ZnCl_2$ all proteins sedimented as aggregates (160). To establish whether the sedimentation was due to protein aggregation or true polymerization, samples were analyzed by electron microscopy. Addition of Mg^{2+} to FtsZ D212N and D212C leads to the formation of typical polymers (Fig. 3). These polymers were stable for at least 60 min, as tested by sedimentation (not shown). Cation-induced polymerization was reversible. Polymer pellets resuspended in the presence of EDTA sedimented only when cations were included in the buffer (not shown). We conclude that removal of the negative charge from position 212 enables polymerization of FtsZ with divalent cations.

Cation-induced polymerization requires GTP-bound FtsZ

Various studies have reported the retention of bound GDP by purified FtsZ with a stoichiometry of ~ 0.5-0.7 mol GDP per mol FtsZ (117, 128). Since the observed cation-

Table 1. Sedimentation of FtsZ D212C and D212N in the presence of divalent cations

Ionic radius[a] (Å)	Polymer sedimentation
0.66	+
0.69	+
0.72	+
0.74	Aggregation
0.80	+
0.99	+
1.12	-
1.34	-

Sedimentation assays were performed as described in the text.
[a] Taken from (150).

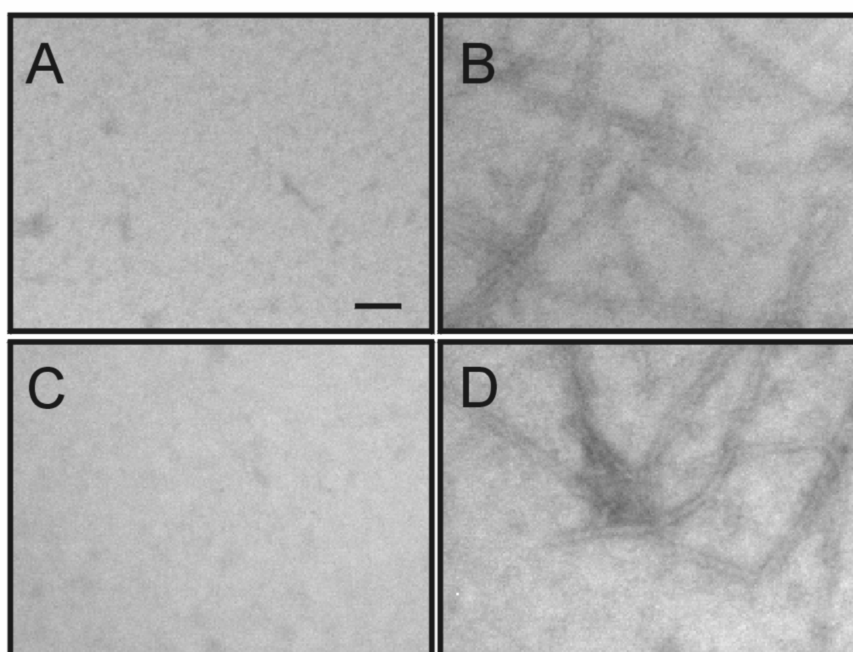

Figure 3. Electron microscopy of wt FtsZ (A) and FtsZ D212C (B), D212E (C) and D212N (D) mutant proteins in the presence of $MgCl_2$. Protein (0.4 mg/ml) was incubated in polymerization buffer with 5 mM $MgCl_2$ for 10 min at 30°C. Samples were analyzed by electron microscopy. bar: 100 nm.

induced sedimentation could be dependent on the retention of nucleotides, purified FtsZ and D212 mutants were analyzed for their nucleotide content. To our surprise, FtsZ D212C and D212N contained predominantly GTP, whereas wt FtsZ and FtsZ D212E contained solely GDP (Table 2). The bound nucleotides (~0.5 mol per mol of FtsZ) could be removed by extensive dialysis (128), but this procedure abolished the polymerization of the wt FtsZ (not shown) and could therefore not be used to determine if nucleotide binding is critical for the cation-induced sedimentation. Next, we tested whether Mg^{2+}-induced polymerization leads to hydrolysis of the GTP bound to FtsZ D212C and D212N. After prolonged incubation with 5 mM $MgCl_2$, only a small fraction of the bound nucleotide was converted into GDP (Table 2). This observation is in agreement with the high stability of the Mg^{2+}-induced polymers. Taken together, these data show that FtsZ D212C and D212N retain significant amounts of GTP after protein purification, which in all likelihood explains the polymerization with cations.

Chemical modification of FtsZ D212C abolishes cation-induced polymerization

The role of the negative charge at position 212 was probed by modification of the FtsZ D212C mutant with the sulfhydryl reagents NEM, MTSEA and MTSES, which are neutral, positively and negatively charged, respectively. Modification of FtsZ D212C abolished cation-induced polymerization (Fig. 4A) while the reagents had no effect on FtsZ D212N (Fig. 4B). Addition of DTT to MTSEA and MTSES labeled FtsZ D212C re-established the cation-induced sedimentation, but it did not restore the activity of FtsZ D212C containing the covalently bound NEM (Fig. 4C). Addition

Table 2. Nucleotides associated with wt FtsZ and FtsZ D212 mutants

Protein		Bound nucleotide (mol/mol FtsZ) GTP		GDP
FtsZ		0		0.74
FtsZ D212E		0		0.65
FtsZ D212C		0.68		0.14
+ $MgCl_2$[a]	15 min		0.49	0.13
	60 min		0.48	0.11
FtsZ D212N		0.49		0.08
+ $MgCl_2$[a]	15 min		0.35	0.10
	60 min		0.28	0.14

Nucleotides were extracted from the protein as described, and identified and quantified by anion-exchange chromatography.
[a] Proteins (1 mg/ml) were incubated in polymerization buffer with 5 mM $MgCl_2$ at 30°C for the indicated times.

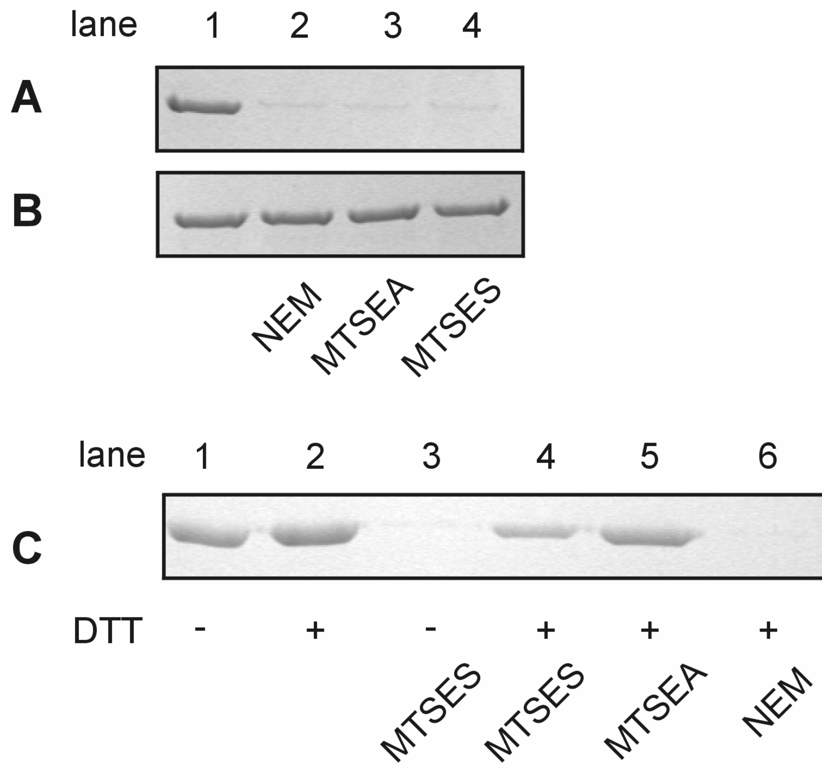

Figure 4. Effect of sulfhydryl reagents on cation induced sedimentation of FtsZ D212C. Sedimentation of FtsZ D212C (A) and FtsZ D212N (B) after incubation for 5 min at 30 °C in polymerization buffer with 5 mM $MgCl_2$. The proteins were modified with nothing (lane 1), NEM (lane 2), MTSEA (lane 3) or MTSES (lane 4). (C) Reversal of cysteine modification with DTT. Sedimentation of FtsZ D212C after incubation for 5 min at 30 °C in polymerization buffer with 5 mM $MgCl_2$ without (lanes 1 and 3) or with 20 mM DTT (lanes 2, 4, 5 and 6). FtsZ D212C was labeled with nothing (lanes 1 and 2), MTSES (lane 3 and 4); MTSEA (lane 5) or NEM (lane 6).

of GTP after labeling did not restore polymerization (not shown). These data indicate that modification of FtsZ D212C with sulfhydryl reagents distorts either the interaction with cations or the interaction of FtsZ monomers in the polymer.

Discussion

In this report we describe FtsZ mutants capable of polymerization without the addition of nucleotides. Removal of the negative charge on position 212 in FtsZ results in protein that purifies with GTP as its predominantly bound nucleotide. The addition of divalent cations allows polymerization of the mutants, suggesting that at the interface of two FtsZ monomers in a functional polymer both GTP and a coordinated metal are required for the formation of a stable structure. The aspartate residue described in this report is conserved throughout all FtsZ proteins known and its position corresponds to a residue that is critical for GTP hydrolysis in tubulins. D212 has been implicated in the coordination of Mg^{2+} at the nucleotide binding site of FtsZ (118). Exchange of this aspartate with a glutamate leads to a dramatic reduction of FtsZ GTP hydrolysis and polymerization activity, indicating that a conserved substitution does not fully restore FtsZ activity. While wt FtsZ and FtsZ D212E purifies with GDP as associated nucleotide, the FtsZ D212C and D212N mutants contain GTP. This surprising observation is most easily explained by a critical role of the negative charge at position 212 in the coordination of Mg^{2+}. In the absence of added Mg^{2+}, wt FtsZ and FtsZ D212E retained the coordinated Mg^{2+} ion that mediates hydrolysis of the bound nucleotide. FtsZ D212C and D212N do not retain Mg^{2+} and therefore contain GTP. In the presence of added GTP and Mg^{2+}, both FtsZ D212C and D212N display GTP hydrolysis activity, although their activity is markedly reduced as compared to the wild type.

The retention of GTP by the FtsZ D212C and D212N mutants permits polymerization in the presence of divalent cations. The size exclusion limit for cation-induced polymerization indicates that the cations bind at a specific site. We propose that this cation-binding site comprises an occupied nucleotide-

binding site on one FtsZ monomer and the T7-loop region on another FtsZ monomer. This requirement for an FtsZ dimer would explain the absence of a cation in the FtsZ crystal structure, which contains one FtsZ monomer per asymmetric unit (77). Cation-induced polymerization of FtsZ D212C can be abolished by modification of the cysteine with neutral, positively and negatively charged sulfhydryl reagents. These reagents do not affect the nucleotide bound to FtsZ D212C and bulk GTP hydrolysis activity (chapter 4). Modification of D212C disrupts either cation binding or the overall interaction of the FtsZ monomers at the nucleotide-binding interface. As previously reported for tubulin (29, 95), nucleotide removal by extensive dialysis results in nucleotide-free, but non-functional FtsZ protein. The tubulin αβ-heterodimer is stabilized by Mg^{2+} and GTP bound at the β-α-interface, also known as the N-site (95). It is likely that the FtsZ multimers found in vivo and in vitro (39, 69, 120, 128) are also stabilized by the presence of nucleotide at the dimer interface. Summarizing, our results indicate that the highly conserved aspartate 212 plays a pivotal role in Mg^{2+} coordination and nucleotide hydrolysis. The D212 mutants provide a good starting point for the further characterization of the Mg^{2+} binding properties of FtsZ.

Acknowledgements

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Experimental procedures

General procedures

Construction and purification of the D212 mutants is described in chapter 4. Protein concentrations were determined using a Bradford assay (17) with a correction factor of 0.82 for the FtsZ/BSA ratio(81).

GTP hydrolysis assay

GTPase activity was monitored by the release of inorganic phosphate (71). FtsZ and mutant proteins (0.1 mg/ml) were incubated in polymerization buffer (50 mM Mes/NaOH; 50 mM KCl; pH 6.5) with 5 mM $MgCl_2$ at 30°C. The reaction was started by the addition of GTP to 1 mM, and hydrolysis was monitored over a period of 30 min.

FtsZ sedimentation assay

FtsZ and D212 mutants (0.2 mg/ml) were incubated for 5 min at 30° C in 50 µl polymerization buffer with divalent cations as indicated. Aliquots (45 µl) were centrifuged using an A-100 18° rotor in a Beckman airfuge at 28 psi for 10 min at room temperature. Pellet fractions were resuspended in 45 µl polymerization buffer, and analyzed by SDS-PAGE and Coomassie Brilliant Blue staining.

Electron microscopy

FtsZ and D212 mutants at 0.4 mg/ml were incubated at 30 °C in polymerization buffer with divalent cations (5 mM). After 5 min, samples were prepared for electron microscopy as described (126) and viewed in a Philips 400T transmission electron microscope.

Nucleotide content analysis and nucleotide removal

Nucleotides bound to FtsZ and D212 mutants were extracted as described (126), and quantified using a MonoQ HR5/5 anion exchange column on an Äkta FPLC system using a linear gradient of 10 mM KH_2PO_4/K_2HPO_4; pH 8.0 to 50 mM KH_2PO_4/K_2HPO_4; 1 M NaCl; pH 7.4 (Amersham Pharmacia Biotech). The column was calibrated with GTP and GDP standards. Nucleotides were removed by dialysis against 50 mM KH_2PO_4/K_2HPO_4; 50 mM KCl; pH 6.5 (128).

Modification of FtsZD212C

Sulfhydryl-specific reagents were used to modify FtsZ D212C. Protein was incubated with methanethiosulfonate-ethylammonium (MTSEA), Methanethiosulfonate-ethylsulfonate (MTSES) (both from Anatrace) and N-ethylmaleimide (NEM) (Sigma) at 2.5 mM; 10 mM and 0.5 mM, respectively. After 10 min labeling at room temperature (66), protein was stored on ice until further use. To reverse the labeling, 20 mM 1,4-Dithio-DL-threitol (DTT) was added and the incubation was continued for 5 min at room temperature.

Chapter 6 - Summary and concluding remarks

Cell division is an important aspect of the bacterial life cycle. In *Escherichia coli*, the Gram-negative model organism, various systems have been identified that ensure proper chromosome duplication and segregation, that identify the division site at the middle of the cell, and that ensure proper division. The cell division process consists of membrane invagination and peptidoglycan synthesis perpendicular to the plane of the membrane allowing septal ingrowth. Closure of this septum finally results in cell separation.

Cell division starts with the formation of a cytoskeletal element known as the FtsZ-ring at the site of division. FtsZ is a protein that is conserved in most bacterial species, and has also been found in some chloroplasts and mitochondria of algae. *In vitro*, FtsZ has been shown to bind and hydrolyse GTP similar to the eukaryotic cytoskeletal protein tubulin, and FtsZ polymerizes into protofilaments that strongly resemble tubulin protofilaments.

The assembly of FtsZ polymers is GTP dependent and dynamic, with disassembly occurring upon GTP depletion. The FtsZ ring functions as a scaffold to which all other cell division proteins localize. The orientation of the FtsZ-ring determines the place of constriction, as evidenced in an *ftsZ* mutant that forms FtsZ spirals instead of rings, and that shows spiral constrictions.

In *E. coli*, various proteins have been identified that interact with FtsZ to regulate ring formation and function. Regulation includes prevention of FtsZ polymerization in the case of DNA-damage (by SulA), stabilization of the FtsZ ring (by ZipA), localization of other cell division proteins (by FtsA) or prevention of localization of cell division proteins (by MinCD).

The work in this thesis focuses on the *in vitro* polymerization properties of FtsZ. An introduction to cell division and FtsZ, as well as a comparison between FtsZ and tubulin, are presented in **chapter 1**.

The experimental work addressed the relationship between GTP-hydrolysis, polymer formation and polymer stability to provide insight in the nature of the FtsZ polymer *in vivo*. In **chapter 2**, the direct relation between polymer stability and GTP presence, and the influence of calcium on FtsZ assembly, are described. Ca^{2+} reduces the rate of GTP turnover and this results in an enhanced stability of FtsZ polymers as studied by a real time light scattering technique. In the presence of Ca^{2+}, FtsZ protofilaments associate laterally to form bundles. These bundles were studied using Differential Interference Contrast video microscopy. FtsZ depolymerization led to a general blurring of the sample, indicating debundling of filaments. Since single filaments are too small to be seen with this technique, the actual depolymerization reaction could not be followed. The influence of Ca^{2+} on FtsZ dynamics, combined with the structural similarity of FtsZ to some known Ca^{2+}-binding proteins, prompted an investigation into the Ca^{2+} binding properties of FtsZ. FtsZ was found to bind Ca^{2+} with a low affinity. The data were interpreted as an influence of Ca^{2+}-binding on the equilibrium between FtsZ polymerization and depolymerization.

The reduction of FtsZ dynamics in the presence of Ca^{2+} was used to study the effects of GTP-analogues on FtsZ polymers, as described in **chapter 3**. FtsZ binds the GTP analogue GTP-γ-S with a similar affinity as GTP, whereas GMP-PNP is not bound. When FtsZ polymers are formed with a limiting amount of GTP, the polymers can be stabilized

by the addition of GTP-γ-S. Since GTP-γ-S is not hydrolyzed by FtsZ, the polymers are stable for a very long time. On the other hand, addition of GDP to preformed polymers results in a decrease of polymer stability and rapid depolymerization. This led to the conclusion that the FtsZ polymer is stabilized by FtsZ subunits that contain GTP (or GTP-γ-S). Analysis of the nucleotide content of FtsZ revealed that over 95% of the FtsZ associated nucleotide is GDP. Only a minor fraction of the FtsZ-associated GDP is exchanged when the polymers are stabilized with GTP-γ-S. This led to the following conclusions: (1) there is no free exchange of the FtsZ-bound nucleotide in the FtsZ polymer; (2) the stabilizing structure that consists of GTP(-γ-S) bound FtsZ is a minor component of the polymer. A model for FtsZ polymer stabilization by a GTP cap, similar to tubulin polymerization, was postulated.

The simultaneous solution of the structures of FtsZ and the α,β-tubulin dimer confirmed the postulated similarity of the proteins. Modeling of these structures into low-resolution images of FtsZ and tubulin polymers pointed at a crucial role for a loop-helix region in the association of FtsZ and tubulin. In the polymers, this T7-loop region contacts the nucleotide-binding site on an adjacent monomer. In tubulin, the difference between the nucleotide-binding site at which GTP hydrolysis does (β-tubulin) or does not (α-tubulin) occur is mediated by a single residue in this region, which is different between α- and β-tubulin. For FtsZ, several mutants in this region have been described that are impaired in GTP hydrolysis. In **chapters 4 and 5**, results of a detailed mutagenesis analysis of this loop region are presented.

The function of several conserved amino acids in the T7-loop was probed by replacement with cysteine. Two aspartate residues in the loop were also replaced with asparagine and glutamate residues. All mutant proteins were purified and characterized *in vitro* as described in **chapter 4**. This revealed that mutation of residues Met206, Asn207, Asp209 and Asp212 severely affects both GTP hydrolysis and polymerization, and that the Asp residues cannot be substituted with Glu. A mutant of Arg214 was less active than wild type FtsZ, but displayed similar polymerization and GTP hydrolysis behaviour. Polymers formed by this mutant were markedly more curved than wild type polymers. Since FtsZ polymers formed with GDP are curved and polymers formed with GTP are straight (80) this pointed at a possible role for the charged Arg residue in Pi release following GTP hydrolysis. Experiments in which the mutant proteins were mixed with wildtype FtsZ revealed that both protein species interact. This led to the conclusion that the T7-loop region plays an important role in GTP hydrolysis, forming the active site by contacting the nucleotide-binding site on an adjacent FtsZ monomer. The FtsZ-FtsZ interaction is not mediated by this loop region alone, since mutant proteins are still able to interact with wildtype FtsZ.

Two of the three mutations of Asp212 resulted in an unusual phenotype: cation-induced polymerization. This is described in **chapter 5**. Wildtype FtsZ and FtsZ D212E purify with GDP as the bound nucleotide, whereas FtsZ D212N and FtsZ D212C purify with bound GTP. This shows that replacement of the negative charge at position 212 allows GTP retention. The GTP containing mutants polymerize in the presence of divalent cations without significant hydrolysis of the bound GTP. Polymerization was observed with cations with an ionic radius up to 0.99 Å indicating a ~1.1 Å size exclusion limit for the cation binding-site. The polymerization was reversible upon cation removal. Modification of the cysteine residue in the FtsZ D212C mutant resulted in loss of the polymerization phenotype but did not affect the bound GTP. This effect proved reversible as well. This work again indicated that the T7-loop region is necessary for the formation of the active site for GTP hydrolysis, with a conserved aspartate residue playing a critical role in cation coordination.

A model for FtsZ polymerization

Based on the results described in this thesis and in the literature, a model for FtsZ polymerization can be postulated. During polymerization, the addition of a FtsZ subunit to the polymer end is coupled to the hydrolysis of GTP on the FtsZ subunit that formed the end of the polymer (see fig. 1). This results in an FtsZ filament that consists of GDP-bound FtsZ with a GTP-bound FtsZ at its ends. Hydrolysis of this GTP by the addition of a new subunit that does not contain GTP, or hydrolysis without addition of a new subunit, results in depolymerization. This model suggests that (1) FtsZ polymers grow vectorially: i.e. in one preferred direction, and (2) FtsZ polymers contain a GTP cap that stabilizes the fast growing filament end like in tubulin. The model does not include release of the hydrolyzed phosphate, which could be a major regulatory determinant for the conformation of the FtsZ polymer. The model is supported by the following observations:

1 - There is a close relation between polymer formation and GTP consumption. GTP depletion results in depolymerization. Both GTP-hydrolysis and polymerization are cooperative processes (81, 102, 104, 128, 155), which indicates that FtsZ-FtsZ association and GTP consumption are closely coupled.

2 - The active site for GTP hydrolysis is formed by two FtsZ monomers ((78), chapters 4 and 5). Mutagenesis of this active site showed how critical the association is for correct polymerization and rapid GTP hydrolysis. It

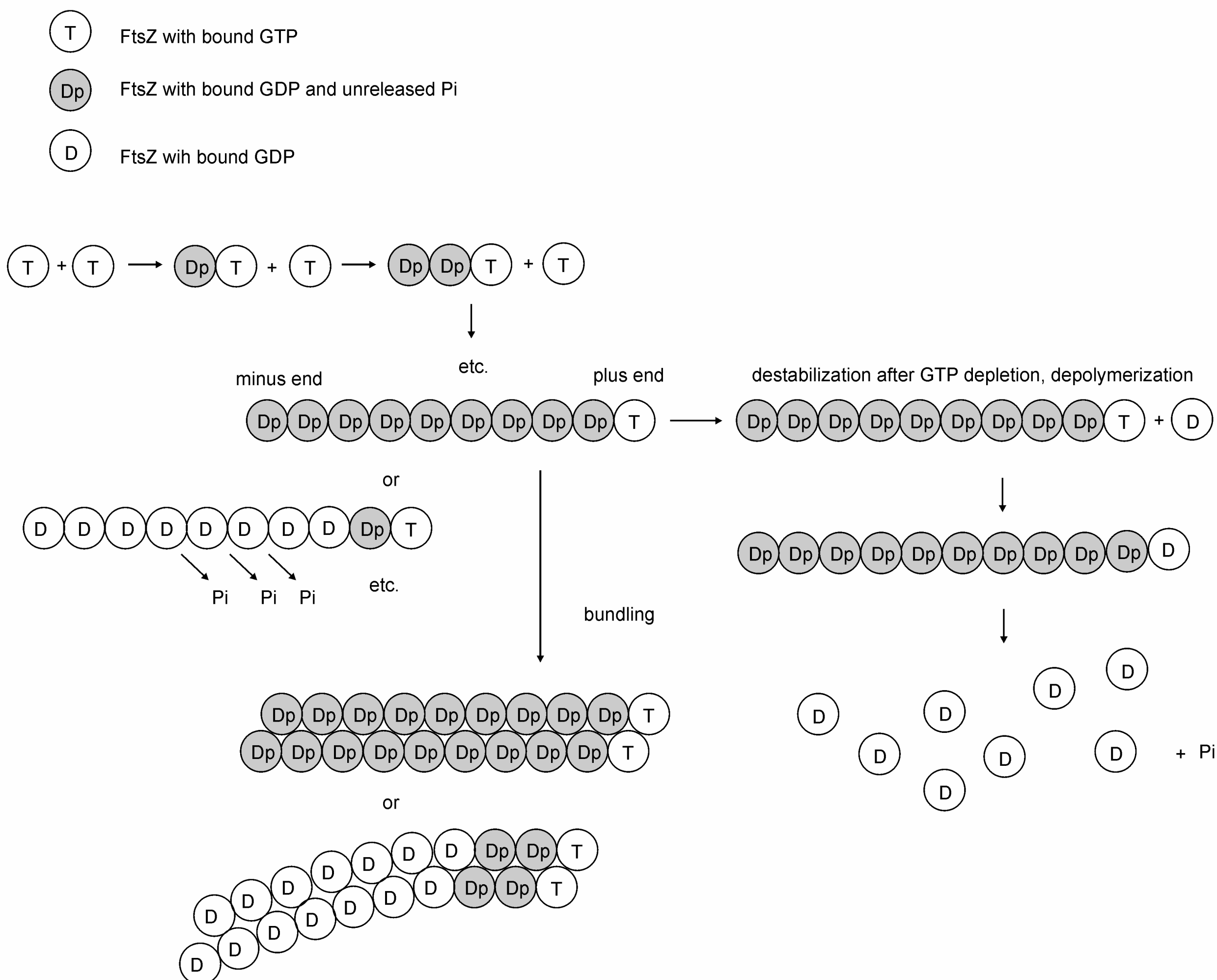

Figure 1. A model for FtsZ polymerization. The model is based on the model described for tubulin and implies polymer growth through addition of GTP containing subunits to the polymer followed by hydrolysis. A GTP containing FtsZ subunit at the polymer end stabilizes the polymer through formation of a GTP cap. This implies polymer vectoriality, as indicated by the plus- and minus-ends. After polymerization polymers can form bundles or sheets. When GTP runs out, addition of GDP containgin subunits to the polymer ends destabilizes the polymers.

would seem illogical that upon polymer formation the active site is assembled without immediate GTP hydrolysis, given the high capacity of FtsZ for GTP turnover.

3 - The work described in chapter 3 revealed that FtsZ polymers contain mostly GDP, which is not exchanged when polymers are stabilized with GTP-γ-S. This provides evidence for rapid GTP hydrolysis giving rise to polymers, which contain GDP that is not readily exchanged with nucleotides in solution, and stabilization of the polymer by a GTP cap. Although this work required high Ca^{2+} concentrations, the polymer formation studied was strictly GTP dependent.

4 - The observation that GTP-γ-S cannot induce FtsZ polymerization, but is capable of polymer stabilization, can be explained by the formation of the active site by two FtsZ monomers. A FtsZ monomer containing GTP-γ-S is capable of adding onto a filament, since the T7-loop of this monomer interacts with the GTP binding site of the final monomer of the filament. The presence of GTP-γ-S at the end of the filament then precludes the addition of more monomers to the filaments, since the interaction between a GTP-γ-S containing FtsZ and the T7-loop of another FtsZ is not correct. This also explains why FtsZ does not polymerize in the presence GTP-γ-S. The destabilizing effect of GDP can also be explained with this model: when a GDP containing FtsZ monomer adds onto the polymer, the GTP bound to the monomer at the end of the filament is hydrolyzed and the filament disassembles since there is no longer a GTP bound FtsZ monomer present at its end.

Some data in the literature challenge the model described above. FtsZ polymerization is not strictly dependent on GTP hydrolysis. Polymers readily form with GTP at conditions that prevent hydrolysis (102, 104) and FtsZ mutants that purify with bound GTP polymerize in the presence of cations without significant GTP turnover (chapter 5). Also, FtsZ polymers have been formed with GDP instead of GTP (51, 80, 101). Assembly with GDP, however, only occurs at high (non physiological) concentrations of cations or DEAE dextran and may therefore not reflect *in vivo* FtsZ assembly. Recently, non-cooperative assembly of FtsZ protofilaments was described that might precede cooperative assembly of FtsZ into laterally associated protofilament sheets and bundles (121). A model to describe this isodesmic assembly implied that the polymers contain GTP and rapidly disassemble upon GTP hydrolysis (121). A problem with this model is that it implies a lag in GTP hydrolysis following polymerization. This has not been observed in a sensitive, real-time assay (128). Another problem with the model is that it implies different kinetics at both ends of the FtsZ polymer. For microtubules a clear difference between fast growing “plus” and slow growing “minus” ends has been observed using microscopy, and it is the plus end of the microtubule which is stabilized by the GTP-cap (37). For FtsZ, there is no evidence that suggests that there is a clear difference between the polymer ends, since nobody has been able to visualize growing FtsZ polymers.

Clearly, more experimental data are required to provide a complete picture of FtsZ polymerization. One of the most important topics in the *in vitro* polymerization dynamics will be the identification of the role of phosphate release upon GTP hydrolysis. Another important challenge is the study of FtsZ polymerization in the presence of purified components of the cell division machinery such as ZipA and FtsA. This will shed more light on the regulation of the FtsZ polymer *in vivo*. More fundamental questions involve the identification of the signal that triggers the assembly of the FtsZ ring, and the identification of the mechanism that generates the force that drives constriction. Constriction could be mediated by the FtsZ ring itself, but could as well involve an, as yet unidentified, factor.