Spatial control of Z ring formation by the Min system in *Escherichia coli*

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Abstract

Bacterial cell division is carried out by the divisome, a multi-protein complex that assembles at the site of division. The first step in the assembly of this complex is the formation of a Z ring at the prospective division site. In rod-shaped bacteria the Z ring is always precisely positioned in the middle of the cell. In *Escherichia coli* this is accomplished by both nucleoid occlusion and the Min system that consists of the three proteins MinC, MinD and MinE. The Min system has been extensively studied in the past few years and the mechanisms behind the actions of the Min proteins have been elucidated a lot. By determining Min protein structures and performing mutagenesis studies many previously unknown binding sites could be revealed, giving new insights on the interactions and dynamics of the system. However, the exact molecular mechanisms behind the functioning of the Min proteins are still not fully understood. This thesis gives a detailed and updated overview of the interactions between the Min proteins by describing the latest findings on the system and how these findings change the existing models and mechanisms.

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Introduction

Cell division or cytokinesis in most bacteria starts with the elongation of the cell and replication of the chromosomal DNA. This is followed by segregation of the DNA and invagination of the cytoplasmic membrane forming a division septum that separates the cell into two daughter cells.

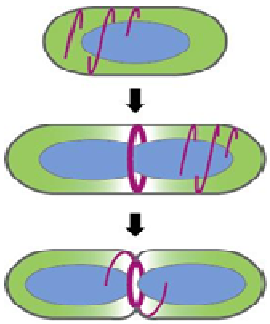

Figure 1: Protofilaments of FtsZ (purple) coalescence into a Z ring in the middle of the cell. *Lutkenhaus, 2007.*

In bacteria the divisome, a multi-protein complex at the prospective division site, functions as the machine for cell division (Adams and Errington, 2009). A crucial protein in this machine is FtsZ, a GTPase and tubulin-like protein, that polymerizes into highly dynamic protofilaments that move along the cell membrane as helix-like waves. This movement is possible because of the fast assembly and disassembly of the polymers (Lutkenhaus, 2007). In rod-shaped bacteria, like *Escherichia coli* and *Bacillus subtilis*, the FtsZ filaments coalescence into a ring just underneath the cytoplasmic membrane in the middle of the cell, called the Z ring (Figure 1). The Z ring is the main part of the divisome and recruits other downstream proteins that mediate in the cytokinesis, e.g. FtsA, ZipA and ZapA. FtsA and ZipA are required for anchoring the Z ring to the membrane and ZapA stabilizes the ring (Adams and Errington, 2009).

During growth, the Z ring is only assembled precisely at mid-cell. Immunofluorescence microscopy and GFP-fusion studies have shown that the Z ring is localized at the cell centre with a standard deviation of just 2.6% and 2.2% respectively (Barák and Wilkinson, 2007). This raised the interesting question how the precise positioning of the divisome complex is regulated. Two systems have been found to control the formation of the Z ring in space and time by preventing assembly at all sites but mid-cell; the Min system and nucleoid occlusion. Nucleoid occlusion prevents division over the nucleoids of the cell, thus preventing a bisection of the chromosome (Cho *et al*., 2011).

The other system, the Min system, prevents division over the cell poles. Loss of the Min system in a bacterium leads to polar divisions that give rise to minicells that lack chromosomal DNA and elongated mother cells that contain two nucleoids (Rothfield *et al.*, 2005). In *E. coli,* three proteins are involved in this system; MinC, MinD and MinE. MinD binds to the membrane and interacts with MinC, resulting in a MinCD complex that inhibits Z ring formation throughout the cell. MinE, a topological specific factor, restricts the activity of the MinCD complex to the cell poles (Lutkenhaus, 2007). Therefore, formation of the Z ring is not prevented by MinCD at mid-cell and the divisome can be formed there.

The exact molecular mechanisms behind the protein interactions in the Min system are not entirely clear yet. However, recent studies shed a new light on some aspects of the system. Especially the determination of the protein structure of the Min proteins made it possible to unravel the interactions between them a bit more. This thesis gives an overview of these recent findings by discussing the proposed models and interaction mechanisms for MinD, MinE and MinC separately, thus giving a detailed description of the Min system.

The *E.coli* Min system is dynamic

The Min system in *E.coli* is not static as was initially thought. GFP-fusion localization studies showed that MinD and MinE oscillate through the cell in such a way that the time-averaged concentration of MinCD becomes the lowest in the middle of the cell. Because of this, Z ring formation is least inhibited at mid-cell and ring formation occurs only at this site (Lutkenhaus, 2007). MinC does not oscillate itself, but binds to membrane-bound MinD, resulting in co-oscillation of MinC with MinD. An *in vitro* study of Loose *et al.* showed that MinD and MinE move as traveling waves on a planar lipid bilayer in the presence of ATP. This supports the dynamic behavior of these proteins *in vivo* (Loose *et al.,* 2008).

In short, each oscillation cycle starts with the binding of ATP to MinD followed by binding of MinD to the membrane. There MinC interacts with MinD, resulting in a MinCD complex that inhibits Z ring formation. The binding of MinD to the membrane always initiates at a cell pole and then extends towards mid-cell. When the MinD binding zone approaches the middle of the cell, MinE appears at the edge of the zone. MinE forms a ring-like structure along the membrane that pushes the MinD binding zone away from mid-cell by displacing MinC from MinD and by stimulating the ATPase activity of MinD several-fold. Because of the resulting hydrolysis of the ATP, MinD-ADP is released from the membrane and the MinD binding zone shrinks back to the cell pole. The E ring does not disassemble until it has reached the cell pole and released all MinCD complexes (Rothfield *et al.*, 2005). Subsequently, the released MinD units can bind a new nucleotide and start to interact with the membrane at the opposite cell pole, thus creating a new MinD binding zone moving towards mid-cell. There, a ring of MinE again disassembles the MinCD complexes, thus restricting the inhibition activity to the cell poles (Figure 2).

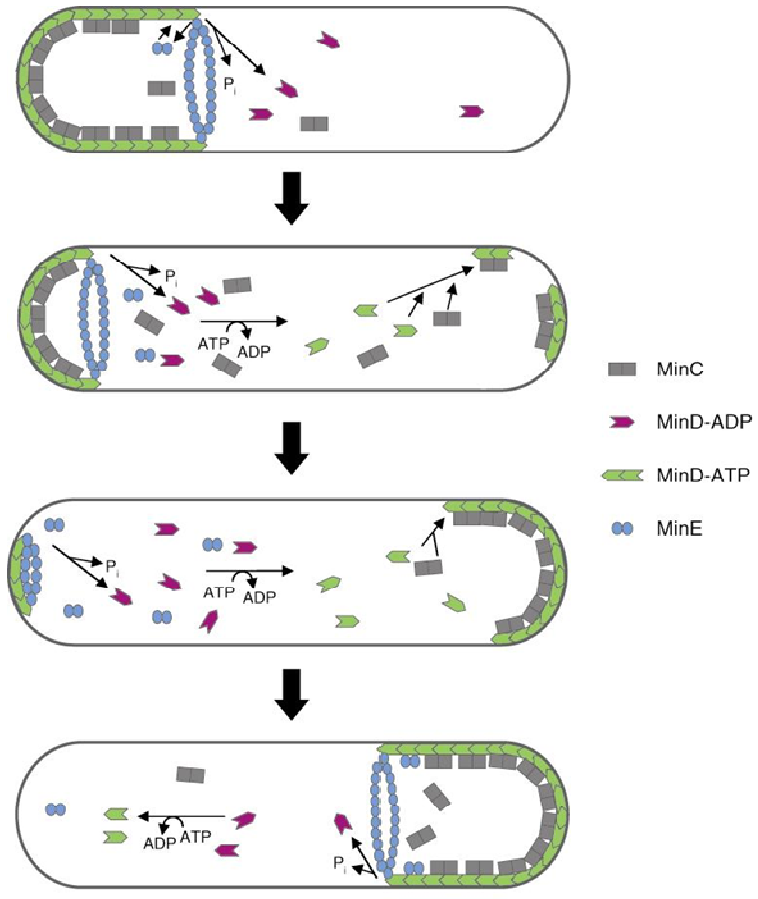

Figure 2: A model of the oscillation in the Min system. MinD binds to the membrane at the cell poles and recruits MinC to form a division inhibiting MinCD complex. The MinD binding zone moves to the middle of the cell, but is pushed back by a ring of MinE that disassembles the MinCD complexes by stimulating ATP hydrolysis. The released MinD molecules bind new ATP and interact with the membrane at the other cell pole and the oscillation cycle repeats. *Lutkenhaus, 2007.*

The periodicity of one oscillation cycle (MinD binding zone extension to mid-cell and its shrinking back to the cell pole) is approximately 40 s at 20˚C (Lutkenhaus, 2007). The oscillation of MinD and MinE is co-dependent, because MinE mutants that could not stimulate the hydrolysis of ATP by MinD showed an increase in oscillation periodicity for MinD (Barák and Wilkinson, 2007). MinE mutants that could not form the E ring were not able to stop the extension of the MinD binding zone towards mid-cell and could therefore not inhibit the MinC activity at the normal division site (Rothfield *et al.*, 2005).

A question that arose from this oscillation model is why the released MinD molecules always bind at the opposite cell pole. One explanation could be that the cell poles contain specific proteins or binding sites that initiate MinD-membrane binding. However, mathematical models have been made of the oscillation of MinD and MinE. These revealed that just diffusional factors, relative membrane affinities of the proteins and rates of nucleotide change by MinD could be the cause of the pole-to-pole oscillation and that no specific proteins are necessary at the cell poles (Rothfield *et al.*, 2005). Moreover, in long filamentous cells the oscillation of MinD does not occur in a pole-to-pole pattern, but occurs in a banded pattern. This banded pattern proves that the poles are not required for nucleating a MinD binding zone. Studies showing that Min oscillation is also present in round cells support this assumption as well. However, it might still be possible that specific marker proteins or sites are present at the cell poles and some positions along the cell membrane to initiate MinD binding (Lutkenhaus, 2007).

MinD

MinD is the component of the Min system that couples the action of MinC and MinE. It recruits MinC and forms a MinCD complex to inhibit cell division throughout the cell. However, because MinE can stimulate the ATP hydrolysis in MinD, the complex is disassembled near mid-cell and division inhibition is restricted to the cell poles.

MinD is an ATPase and belongs to the great MinD/ParA/Mrp deviant Walker A motif family (Park *et al.*, 2012). The members of this family are also called Walker A cytoskeletal ATPases (WACAs), because some of them seem to assemble into polymers. There is not much sequence homology between the WACA proteins, but their structures show a lot of similarities (Lutkenhaus, 2007).

Dimerization

All members of the WACA family undergo ATP-dependent dimerization and contain variants on the deviant Walker A motif: KxxxxGKT. This motif contains two lysines, the first one of them being the ‘signature’ lysine (in MinD: K11) that seems to be required for dimerization of the proteins. ATP-dependent dimerization has been observed for MinD as well, suggesting it is present as a dimer. Importantly, the dimerization is involved in the binding of MinD to the cytoplasmic membrane (Lutkenhaus, 2007).

MinD interacts with the membrane through a membrane targeting sequence (MTS) at its C-terminus, consisting of an amphipatic helix of 10 amino acids (Wu *et al.*, 2011). This helix is responsible for the membrane binding; without this sequence MinD cannot bind the membrane. The helix in *E. coli* MinD preferentially interacts with anionic phospholipids and inserts its large hydrophobic residues into the bilayer (Lutkenhaus, 2007). Remarkably, GFP fused to one copy of the MTS does not bind the membrane, but GFP fused to a tandem repeat does (Wu *et al.*, 2011). This suggests that MinD can only associate with the membrane as a dimer (or oligomer).

It is not clear whether the ATP-dependent dimerization of MinD takes place before MinD interacts with the membrane or whether binding of MinD to the membrane enhances dimer formation. *In vitro* studies revealed that MinD dimerizes more easily when the amphipatic helix is removed, suggesting that the membrane interaction is needed for sequestering the helix (Lutkenhaus, 2007). In this case the membrane would promote dimerization of MinD. Fluorescence resonance energy transfer (FRET) studies between labeled MinD proteins support this assumption as they revealed that dimerization is promoted by the presence of vesicles (Lutkenhaus, 2007). Thus, MinD seems to undergo surface-assisted ATP-dependent dimerization, although other deviant Walker A motif family members do polymerize in solution without the assistance of a phospholipid bilayer.

Protein structure of MinD

The structure of an *E. coli* mutant dimer has recently been determined (Wu *et al.*, 2011) (Figure 3). Wu *et al.* were able to crystallize MinDΔ10-D40A in the presence of ATP and to resolve its structure at 2.4 Å resolution. The last 10 residues of the C-terminus of MinD (the MTS) were deleted, since this resulted in a higher solubility of the protein. The D40A mutation prevents the hydrolysis of ATP, since the aspartate is required for ATPase activity, but does not disrupt the binding of MinC and MinE.

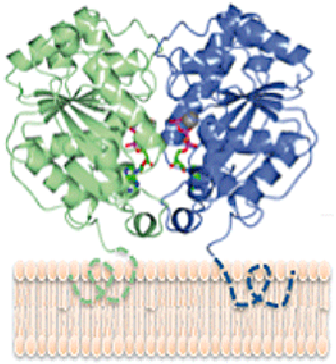

Figure 3: Structure of MinDΔ10-D40A dimer. The monomers are colored blue and green. The possible position of the last 10 truncated residues is shown as a dashed line. *Wu et al., 2011.*

The obtained structure revealed a nucleotide sandwich dimer, similar to the structure of other deviant Walker A motif family members, like Soj. The position of the last 10 truncated residues of the C-terminus was predicted by looking at the orientation of the residues just before the truncation. The structure of MinDΔ10-D40A reveals that the residues preceding the truncation are positioned in a helix that extends to the bottom of the structure. Since the membrane binding amphipatic helix lies directly past these residues in wild-type MinD, it is clear that the bottom face of the structure must be near the membrane (Wu *et al.*, 2011).

ATPase activity

A characteristic of members of the deviant Walker A motif family is that they all dimerize in an ATP-dependent manner and that the 'signature' lysine in the deviant Walker A motif (in MinD: K11) is critical for this dimerization (Park *et al.*, 2012). In MinD this lysine has interactions with several residues in helix 7, especially D152, and in a MinD dimer the lysine interacts with ATP on the other subunit (Lutkenhaus, 2007). When MinE is recruited by a MinD dimer it stimulates the ATPase activity of MinD and causes hydrolysis of ATP, resulting in the disassembly of the complex and release from the membrane. The exact mechanism of the ATP hydrolysis and how MinE stimulates this is not clear yet.

It is known that efficient hydrolysis requires two things: i) activation of a water molecule and ii) neutralization of the negative charge developing during the transition state (Park *et al.*, 2012). The positively charged lysine residue can neutralize the negative charge. Furthermore, MinD has an aspartate residue (D40) that is thought to activate a water molecule for nucleophilic attack of the γ-phosphate of ATP. The aspartate has shown to be important for ATP hydrolysis, because a MinD-D40A mutant can bind MinC and

MinE but lacks ATPase activity. Still, MinE is required for ATPase activity, since ATP is not hydrolyzed when MinD has no interaction with MinE (Park *et al.*, 2012).

In 2004, Ma *et al.* found that MinE interaction with residue D152 of MinD is important for efficient ATPase activity, since MinD response to MinE is reduced when D152 is mutated. They suggested a model in which MinE competes with the 'signature' lysine K11 for binding to D152 (Ma *et al.*, 2004). When MinE is recruited by MinD it binds D152, so that the lysine becomes free and is able to interact with the ATP, thus promoting hydrolysis.

Wu *et al.* (2011) discard this model, because the structure of MinD revealed that after dimerization K11 does not interact with D152 but with the phosphates of ATP in the other monomer. They suggest that K11 is already released from D152 during dimerization, so that MinE cannot compete with it anymore when it is recruited by the MinD dimer. Thus, the structure shows that dimerization and not MinE is responsible for the release of K11 from D152.

Among the members of the deviant Walker A motif family one asparagine is conserved (in MinD: N45) (Park *et al.*, 2012). In one of these members, Get3, the equivalent asparagine seems to be involved in the ATPase reaction. Therefore, the N45 in MinD could be important for ATP hydrolysis as well. Early studies already revealed that a MinD-N45A mutant is able to dimerize, bind ATP and the membrane and activate MinC. Recently, Park *et al.* (2012) tested the response of MinD-N45A for MinE. This study showed that MinE binds MinD, but that ATPase activity is poorly stimulated. The N45 residue in MinD is thus critical for stimulation of ATPase activity by MinE.

The mechanism for the ATP hydrolysis in MinD is still not clear. However, certain residues in MinD, like K11, D40, N45 and D152, seem to be important for this activity. It is also important to mention that hydrolysis only occurs when MinD is bound to the membrane. The hydrolysis of ATP in non-membrane binding mutants of MinD is not stimulated by MinE (Park *et al.*, 2012). This indicates that membrane binding causes structural changes in MinD, uncovering an accessible binding site for MinE. This hypothesis will be further discussed in the section about MinE (see *Conformational changes upon MinD binding*).

The MinE binding region

The region of MinD that is bound by MinE was very unclear until recently. Especially a study of Wu *et al.* (2011) revealed many MinD residues that are involved in MinE binding, making it possible to define the MinE binding region.

In earlier studies the residue D152 was thought to be critical for MinE binding (Ma *et al.*, 2003). A yeast two-hybrid study of MinD and $MinE^{1-31}$ showed that the interaction between these two proteins decreases dramatically in a MinD-D152A mutant, suggesting that D152 is required for MinE interaction. Residues 1-31 in MinE represent the domain that counteracts the MinCD division inhibitory activity (Park *et al.*, 2011). In addition, Wu *et al.* (2011) recently examined the interaction between MinD and full length MinE (and $MinE^{1-31}$ as well) in a bacterial two-hybrid study as this system seemed to be more useful than a yeast two-hybrid study. Their study showed that a MinD-D152A mutant has reduced interaction with full length MinE (~50% reduction) and $MinE^{1-31}$ (~90% reduction). Still, the D152 residue does not seem to be critical for MinE binding like was thought before, but it thus does affect the interaction with MinE and even more the reaction with $MinE^{1-31}$.

To find more residues in MinD that are involved in the MinE binding site Wu *et al.* (2011) performed a random mutagenesis study in which they screened for mutants of MinD that fail to bind MinE, but can still bind MinC. They wanted the MinD mutants to be able to activate MinC, because MinC and MinE appear to have overlapping binding sites

and they wanted to identify residues that affect just MinE. The screen revealed four of these mutants: MinD-E53K, -D192Y, -M193L and –G224C. Thus the residues E53, D192, M193 and G224 in MinD are important for MinE interaction, but do not affect MinC interaction. The structure of MinD reveals that these four residues are located at the dimer interface, so Wu *et al.* suggested that MinD dimerization is necessary for creating a suitable binding site for MinE (Wu *et al.*, 2011).

To investigate this binding site further, they performed a site-directed mutagenesis study. Another nine MinD mutants were found that do not bind MinE but do bind MinC: L48K, D67R, V147E, L194D, D198R, I202D, L218E, S221R and N222A. In a bacterial two-hybrid study these 9 mutants and the 4 mutants found by random mutagenesis were tested for interaction with MinE. All of them showed no MinE interaction, except for the MinD-D67R mutant, that seems to weakly bind MinE. To better determine the surface of MinD for interaction with MinE, another screen was done, but for mutants that do not fail in MinC or MinE binding and are thus not involved in the MinE binding region. Forty-two mutants were generated and these and the residues involved in MinE binding were mapped on the structure of MinD (Figure 4). The mapping again confirms that the MinE binding site is created only after dimerization, since all 13 residues involved in MinE binding are positioned at the dimer interface. Thus, the region of MinD that binds MinE extends from the bottom to the top of the dimer interface (Wu *et al.*, 2011).

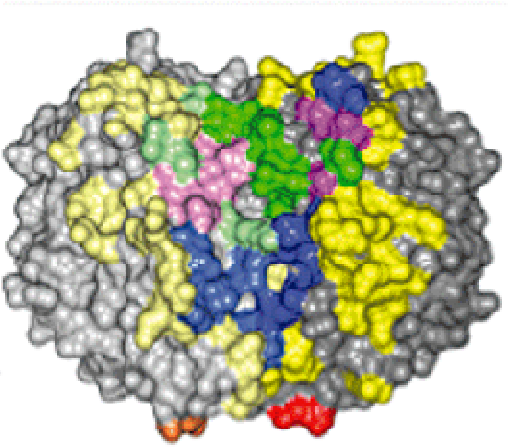

Figure 4: Structure of MinDΔ10-D40A dimer with mapped residues. The different colors represent residues that are involved in MinE binding (blue), MinC binding (green) or both MinE and MinC binding (pink) and residues that are not involved in binding of MinE or MinC at all (yellow). The mapping shows that the binding site of MinE and MinC overlap and lie at the dimer interface of MinD. *Wu et al., 2011.*

The MinC binding region

The MinC binding region of MinD has been investigated as well. During the examination of the MinE binding site of MinD several mutants were found that did bind MinE, but not MinC (Wu *et al.*, 2011). Especially residues L157, G158 and A161 in helix 7 of MinD seem to specifically interact with MinC. To define the binding site, Wu *et al.* (2011) performed a random and site-directed mutagenesis of MinD and screened for more MinD mutants that are able to dimerize and bind MinE, but are unable to interact with MinC. This screen revealed 9 extra residues that are important in MinC-binding: R44, V57, Q90, T91, R92, R151, I159, A161 and P173. The R44 residue lies in a small region, designated the MinD-box, that is highly conserved among MinD proteins, but not among the members of the deviant Walker A motif family (Zhou and Lutkenhaus, 2004). Zhou and Lutkenhaus found more residues in this region of MinD that affect the binding of MinC and therefore suggest that the MinD-box is involved in MinC binding.

All residues involved in MinC binding and forty-two residues that have proven not to be involved in MinC or MinE binding (see *The MinE binding region*) were mapped on the structure of MinD (Figure 4). The mapping shows that the MinC binding site lies at the upper half of the dimer interface of MinD, indicating that ATP-dependent dimerization and

membrane binding of MinD are required to create the binding site and to expose it to the cytosol, where MinC can easily interact with it (Wu *et al.*, 2011). Furthermore, many earlier studies suggested that MinE and MinC have overlapping binding sites, because MinE displaces MinC. The mapping of the MinC and MinE binding residues confirms the overlap and also shows that the binding site for MinE is much more extended along the dimer interface than the binding site for MinC (Wu *et al.*, 2011).

MinE

In the *E. coli* Min system, MinE functions as the topological specificity factor, since it restricts the inhibitory activity of MinCD to the cell poles (Lutkenhaus, 2007). The protein co-oscillates with MinD and forms an E ring at the edge of the MinD binding zone, thereby stimulating ATP hydrolysis and disassembly of the MinCD complex.

MinE is a protein of 88 residues, that is found as a dimer in the cell. Each monomer consists of two domains; residues 1-31 form the N-terminal domain and residues 32-88 form the C-terminal domain. Early studies showed that each domain has its own functions (Lutkenhaus, 2007). The N-terminal domain contains the region that is responsible for inhibition of the MinCD complex and is therefore called the anti-MinCD domain. Since MinCD inhibition is correlated to MinE binding to MinD and activation of ATPase activity, the anti-MinCD domain was expected to be involved in MinD binding. The C-terminal domain of MinE was thought to be responsible for dimerization of the protein and for its topological specificity. However, the dependency of the two domains on each other was not understood and the functions remained quite unclear (Lutkenhaus, 2007).

Recent research, especially the determination of the structure of MinE, shed a new light on the functions of the MinE domains. It has been shown that the N-terminal region does not only contain a MinD binding region (the anti-MinCD region), but also a region for binding directly to the membrane, suggesting that this domain plays a role in the topological specificity of MinE (Hsieh *et al.*, 2010; Park *et al.*, 2011). The functions of the C-terminal domain have changed as well; this domain functions in dimerization, but is now also thought to play a role in the onset of conformational changes in MinE upon MinD-binding (see *A final model for MinD-MinE interaction?*). In recent models of the Min system, MinE undergoes conformational changes when it reaches MinD, thus making the anti-MinCD and membrane binding regions more accessible for interaction (Park *et al.*, 2011).

Protein structure of MinE and the MinD-MinE complex

The structure of MinE was determined in several studies. A NMR study of King *et al.* revealed the structure of only the C-terminal domain of MinE (King *et al.*, 2000). This structure shows a dimer, consisting of a four-stranded anti-parallel β-sheet and two α-helices (Barák and Wilkinson, 2007) (Figure 5A). The structure of the N-terminal domain was not determined, but several tests predicted that it would have a helical conformation.

More recently, the structure of full length MinE from different organisms was determined (Ramos *et al.*, 2006; Kang *et al.*, 2010, Ghasriani *et al.*, 2010). Kang *et al.* (2010) were able to crystallize *Helicobacter pylori* MinE and obtain its structure at 2.8 Å resolution. This structure revealed that *H. pylori* MinE exists as a dimer and that each monomer is composed of a long α-helix (residues 35-50) and three β-strands forming an anti-parallel β-sheet (β1(19-26), β2(54-59), β3(67-74)). The presence of the β1-strand shows that the N-terminal domain is not in the helical conformation that was predicted by

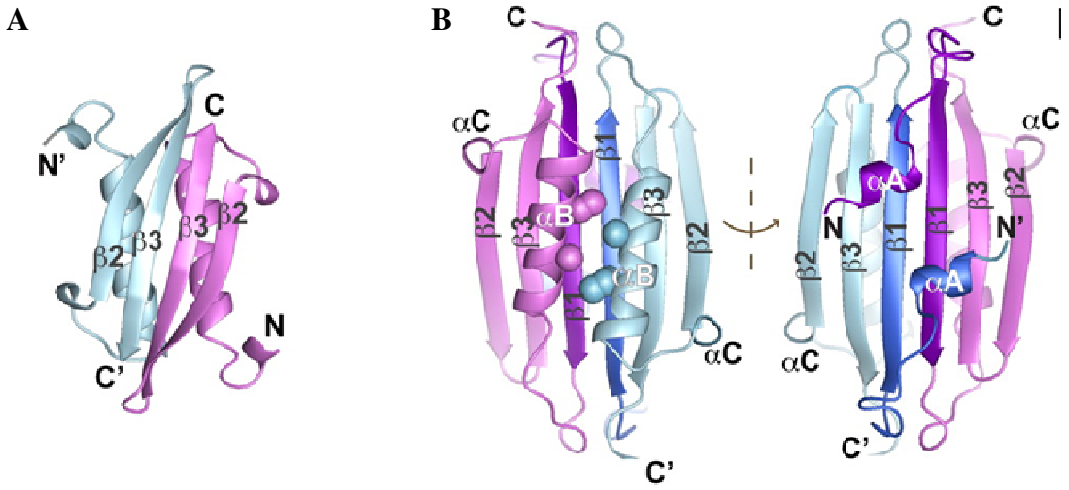

Figure 5: A) Structure of a truncated MinE$^{32-83}$ (C-terminal domain) dimer from *E. coli*. A four-stranded anti-parallel β-sheet is seen. **B)** Structure of full length MinE dimer from *N. gonorrhoeae*. A six-stranded anti-parallel β-sheet is seen. The N-terminal domain of each monomer consists of β-strand β1 and the short helix αA at the back site of the protein. *Ghasriani et al., 2010 (adapted).*

King *et al.*. The structure of Kang *et al.* confirms the findings of Ramos *et al.* (2006), who determined the structure of full length *Neisseria gonorrhoeae* MinE by solution NMR and showed the presence of a β-strand in the N-terminal domain as well.

Kang *et al.* were unable to determine the structure of the first ~10 residues of *H. pylori* MinE, but the structure of full length *N. gonorrhoeae* MinE (Ghasriani *et al.*, 2010) revealed that these residues are positioned in a short α-helix perpendicular to and behind the six-stranded β-sheet of the dimer (Figure 5B). Thus, the structures of MinE show that the N-terminal domain of each monomer consists of a short helix and one β-strand and that the C-terminal domain consists of one long helix and two β-strands.

In addition to the MinE structures, Park *et al.* (2011) determined the structure of two *E. coli* MinD-MinE complexes to investigate the binding interactions. Crystals were obtained of MinDΔ10-D40A bound to his-tagged MinE$^{12-88}$-I24N (Figure 6). The proteins

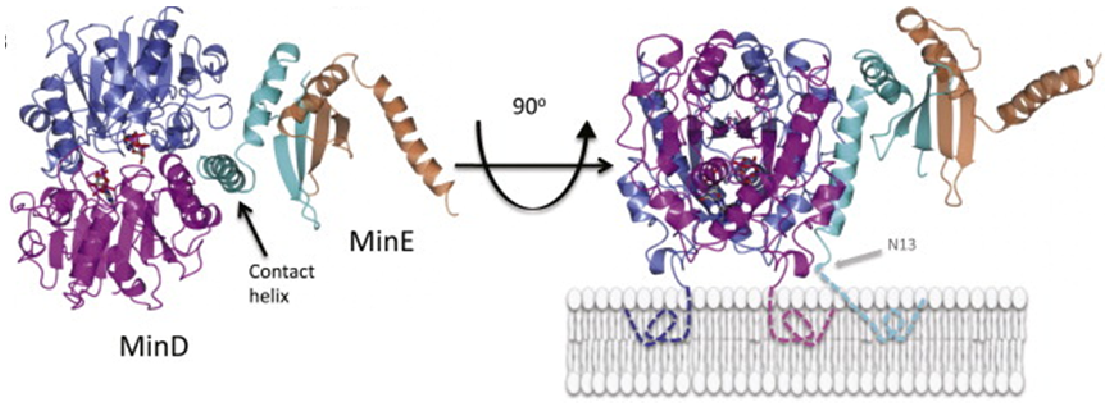

Figure 6: Structure of MinDΔ10-D40A in complex with his-tagged MinE$^{12-88}$-I24N (both dimers). The orientation on the left shows clearly that MinE binds at the dimer interface of MinD with residues 13-26 in a helical conformation. The orientation on the right shows that the first 11 residues of MinE (that were truncated) point toward the membrane. Therefore, Park *et al.* (2011) predict that these residues function as MTS and they are drawn as a membrane binding helix (dashed line) corresponding to the short helix αA in Figure 5B. *Park et al., 2011.*

were point mutated and the last 10 residues of MinD (the MTS) and the first 11 residues of MinE were removed to prevent the formation of aggregates, which occurred during crystallization of the wild-type proteins. Since earlier studies showed that a N-terminal peptide of MinE can bind MinD, Park *et al.* also determined the structure of MinDΔ10-D40A in complex with $MinE^{12-31}$ (Park *et al.*, 2011).

The structures of the MinD-MinE complexes reveal a different conformation for the N-terminal region than the MinE structures. In the MinD-MinE complex residues 13-26, that correspond to the β1-strand, are in a helical conformation. This anti-MinCD helix fits in the MinD dimer interface, a region of MinD that contains many residues involved in MinE-binding (see *The MinE binding region*). In the complex, the C-terminal domain of MinE forms a four-stranded β-sheet in the same way as was seen by King *et al.* (2000) in the structure of the MinE C-terminal domain (Figure 5A). Thus, the structure of MinE in complex with MinD is consistent with a four-stranded β-sheet and not a six-stranded β-sheet (Park *et al.*, 2011). This strongly supports recent studies that suggest that MinE changes its conformation for interaction with MinD (see *Conformational changes upon MinD binding*) .

Dimerization

MinE is almost always present as a dimer. The structures of MinE show that the dimer interface is formed by interactions in two parts of the monomer. First, the C-terminal helices of two monomers are kept together by hydrophobic interactions between their highly conserved hydrophobic residues. Second, the β1-strands interact with each other in an anti-parallel way, thus creating a six-stranded anti-parallel β-sheet (Kang *et al.*, 2010). The involvement of the N-terminal domain (β1-strand) in dimerization was surprising, because earlier studies showed that N-terminally truncated MinE can still form dimers, indicating that only the C-terminal domain is responsible for dimerization (Ghasriani *et al.*, 2010). However, recent models of MinD-MinE interaction give an explanation for this by suggesting that MinE undergoes conformational changes that also affect the dimer interface (see *A final model for MinE action?*).

Several studies suggested that MinE dimerization is required for full activity in MinD displacement (Lutkenhaus, 2007). Five *E. coli* MinE mutants have been found that are unable to form E rings during oscillation; A18T, L22R, I25R, D45A, V49A. The equivalent residues in *H. pylori* MinE are all located at the dimer interface of the structure except for A18 (Kang *et al.*, 2010). Kang *et al.* therefore suggest that these residues are involved in dimerization and that the MinE mutants are unable to form E rings, because of weakened dimer interactions. This supports the assumption that proper dimerization is required for full functioning of MinE (including E ring formation). However, other studies showed that some of these residues could play a role in MinD-binding (A18, L22, I25) or membrane-binding (D45, V49) of MinE instead of dimerization, thus explaining the reduced activity of the mutants in another way (Ghasriani *et al.,* 2010; Hsieh *et al.*, 2010).

Direct membrane-binding of MinE

Until recently, it was thought that the C-terminal domain of MinE is responsible for the topological specificity and the possibly related dimerization of the protein. The structures of full length MinE revealed that the N-terminal domain also plays an important role in dimerization and might thus be responsible for topological specificity as well (Kang *et al.,* 2010). However, the topological specificity might not only depend on the dimerization and the way in which MinE obtains this specificity is not clear yet.

Hsieh *et al.* (2010) examined the interactions between MinE and the cytoplasmic membrane. They show that MinE is not only recruited to the membrane by MinD, but can

also directly interact with it and found clues that this membrane-interaction plays a role in topological specificity.

The direct interaction of MinE with the membrane was proven in *in vitro* sedimentation studies using MinE and liposomes from *E. coli*. The two domains of MinE were also tested separately; $MinE^{1-31}$ showed a 6-fold higher sedimentation with liposomes than wild-type MinE and $MinE^{32-88}$ showed no sedimentation at all. This means that the membrane-binding region (MTS) lies in the N-terminal domain and that the C-terminal domain in some way inhibits direct membrane-binding.

Hsieh *et al.* also provide evidence that 8 positively charged residues (R10, K11, K12, K19, R21, R29, R30 and R31) in the N-terminal domain are responsible for the membrane-binding through electrostatic interactions. Especially residues R10, K11 and K12 are important for membrane-binding, because a triple MinE mutant of these three residues does not bind the membrane. The involvement of electrostatic interactions was shown in sedimentation assays in which the interaction of $MinE^{1-31}$ with the head groups of anionic phospholipids was tested at different salt concentrations. The higher the salt concentration, the more MinE interaction with the lipids was reduced, indicating that electrostatic forces are involved in the interaction.

Furthermore, liposomes of different lipid composition were tested. These tests revealed that $MinE^{1-31}$ interacts more with the membrane when the amount of phosphatidylglycerol and cardiolipin is increased. Interestingly, the membrane at mid-cell is enriched in cardiolipin at certain stages during the cell cycle (Mileykovskaya and Downhan, 2009). This provides an explanation for topological specificity via membrane-binding (Hsieh *et al.*, 2010). In addition, the interaction of MinE decreases when the amount of cationic phospholipids is raised, thus confirming the involvement of the positively charged residues in membrane-binding.

Hsieh *et al.* also suggest that direct membrane-binding of MinE is critical for correct functioning of the protein. It is known that a D45A or V49A mutant of MinE is unable to form E rings. Sedimentation assays show that these mutants do not interact with the membrane, indicating that membrane-binding is critical for E ring formation (Hsieh *et al.*, 2010). Other tests revealed that these mutants disturb the MinE oscillation cycles, thus again supporting the idea that direct membrane interaction is necessary for good functioning of MinE. Moreover, these mutants support the idea that the C-terminal domain in some way regulates the membrane-interaction, since the mutations are not in the membrane-binding N-terminal domain (Hsieh *et al.*, 2010).

Another MTS in MinE is suggested by Park *et al.* (2011). They suggest that not the positively charged residues 10-12, but the N-terminal short amphipatic helix (residues 1-8) is responsible for the membrane-binding of MinE. This short helix contains large hydrophobic conserved residues and therefore likely binds to the membrane. To test this possibility, the hydrophobic residues in the helix were substituted for charged ones in a MinE-I25R mutant. This mutant has shown to directly bind the membrane, but when the residues in the helix are substituted, MinE is unable to interact with the membrane. Thus, the short N-terminal amphipathic helix can also be a good MTS.

The short helix was not present in the determined structures of the MinD-MinE complex. Nevertheless, the position of the helix can be predicted by looking at the residues next to it that are present in the structure. These residues, including two glutamates, extend to the bottom of the structure in the direction of the membrane, thus suggesting that the helix is oriented near the membrane (Park *et al.*, 2011) (Figure 6). This supports the idea that the helix functions as MTS.

Conformational changes upon MinD binding

The structures of full length MinE show that the anti-MinCD domain has a β-conformation (β1-strand) (Kang *et al.*, 2010; Ghasriani *et al.*, 2010). However, the dimer interface of MinD that is suggested to contain the MinE-binding residues forms a cleft in which a helix would fit well. Therefore, it seems likely that MinE changes its anti-MinCD conformation to a helical one to fit the MinD dimer (Wu *et al.*, 2011). Furthermore, in many studies the C-terminal domain has shown to affect the binding ability of MinE without being involved directly in the interactions. Thus, this domain might possibly be involved in the onset of conformational changes of MinE.

Several residues of MinE have been found to be involved in MinD-binding, especially A18, K19, R21 and L22 (Ghasriani *et al.,* 2010). Residues 18-22 are not very accessible in the MinE structure. In addition, other residues in the region 1-22 have also shown to participate in MinD-binding, but are buried in the hydrophobic dimer interface of MinE. Solution NMR spin relaxation experiments revealed that many residues in this region show motion on the microsecond to millisecond time-scale, thus confirming the conformational flexibility of this region (Ghasriani *et al.,* 2010). Ghasriani and Goto (2011) therefore propose that MinE undergoes dynamic changes to make the anti-MinCD region accessible, either by dissociation of the dimer into monomers or by conformational changes. To support the latter, they show that changing the β1-strand into a helix would bring residues 18-22 on the same site of the helix, so that they would easily be able to interact with MinD (Ghasriani and Goto, 2012).

Hsieh *et al.* (2010) also speak of conformational changes in MinE. However, they rather suggest that these changes occur to make the membrane-binding region of MinE accessible (according to them especially residues 10-12) instead of the anti-MinCD region. After freeing the membrane-binding region by conformational changes, MinE would be able to interact with anionic phospholipids of the membrane (especially at mid-cell) and formation of the E ring would be enhanced in a yet unidentified way (Hsieh *et al.*, 2011).

The structure of the MinD-MinE complex proves that the anti-MinCD region changes its β-strand into an α-helix that fits in the cleft at the MinD dimer interface. Meanwhile, the C-terminal domain forms a dimer with a four-stranded β-sheet (Park *et al.*, 2011). Although this greatly supports the idea that MinE changes conformation upon MinD-binding, it does not explain the mechanism behind it.

Based upon mutant studies, Park *et al.* (2011) suggest that MinD and MinE have to 'sense' each other when MinE approaches MinD to induce the conformational changes in MinE that free the MinD-binding region and MTS (according to them residues 1-8). Mutations in I24, L22 and I25, enhance membrane-binding, while these residues are not present in the MTS. Park *et al*. assume that mutation of these hydrophobic residues weakens the dimer structure thus giving it a more open conformation. Because of this the binding regions are more accessible and the MTS can interact with the membrane. Thus, the MinD-sensing step that normally would change the conformation is skipped (Park *et al.*, 2011).

A final model for MinE action?

A very recent model for the interaction between MinE and MinD is proposed by Park *et al.* (2012). Although most of the studies mentioned before support the model, one should still keep in mind that not every part of it is completely understood. For instance, in the following model Park *et al.* assume that the MTS lies in the N-terminal amphipatic helix (residues 1-8), while Hsieh *et al.* (2010) suggest that 8 positively charged residues, including residues 10-12, are responsible for interaction with the membrane through electrostatic interactions.

In the model proposed by Park *et al.* MinE is present in the cytosol as a six-stranded dimer. The C-terminal domain is involved in the dimerization and sequesters the anti-MinCD region and MTS (the short amphipatic helix). When MinE reaches a membrane-bound MinD dimer, the two proteins 'sense' each other thus inducing conformational changes in MinE. One β1-strand of the MinE dimer becomes more accessible and binds at the MinD interface in a helical conformation (approximately residues 9-22) thus displacing MinC. The MTS (residues 1-8) at the end of this anti-MinCD helix is in this way positioned near the membrane and interacts with it. The other freed β1-strand does not bind MinD, but independently binds the membrane via the MTS. The anti-MinCD domain that has bound MinD stimulates ATP hydrolysis of both ATPs in the MinD dimer, resulting in dissociation of MinD from the membrane. The MinE protein then has two options: either it releases the membrane and falls back into a six-stranded dimer or it graps another nearby MinD dimer with the second β1-strand and stays in its four-stranded conformation (Park *et al.*, 2012).

This grabbing of new MinD dimers after hydrolyzing another one could also give an explanation for the movement of the E ring to the cell poles during oscillation. Park *et al.* (2011) compare the travelling of MinE from one MinD to another MinD to the travelling of Tarzan on vines through the jungle. In this analogy MinE is compared to Tarzan and MinD is illustrated by the vines. Every time a MinD dimer dissociates from the membrane (a vine falls from the trees) MinE grabs another one with its free β1-strand (the free arm of Tarzan that has not yet grabbed a vine) (Park *et al.*, 2011) (Figure 7).

In another study, Park *et al.* (2012) provided more evidence for the Tarzan travelling model of MinE. The model suggests that both ATPs in the MinD dimer can be hydrolyzed by binding of just one MinE peptide, because the other is bound independently to the membrane without interaction with MinD. This suggests asymmetric activation of the MinD ATPase. Park *et al.* showed that the hydrolysis of ATP in one subunit of MinD can induce conformational changes in the second subunit, thus stimulating ATP hydrolysis there as well. Residue R21 in MinE is thought to be important in this mechanism, because this residue does only interact with residues of one MinD subunit. These asymmetric

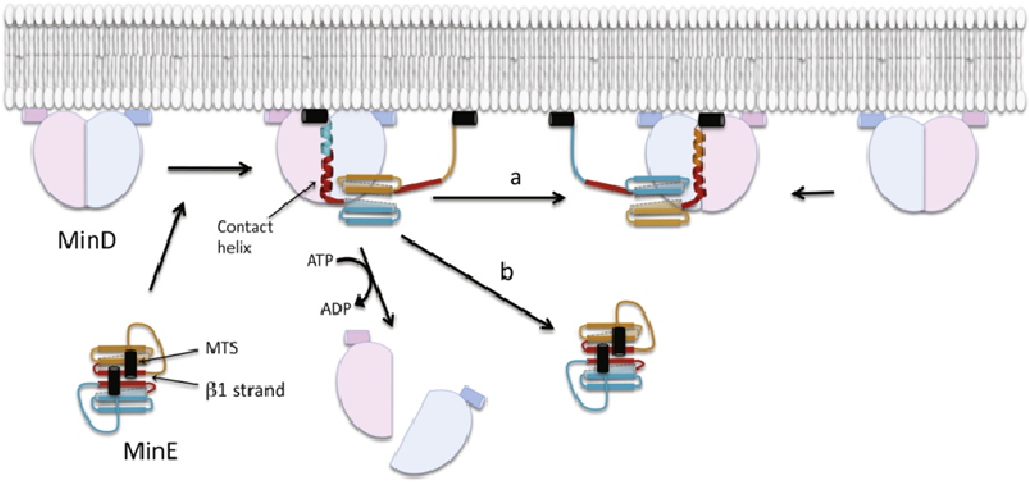

Figure 7: The 'Tarzan travelling on vines' model of MinE. MinE and MinD sense each other and the conformation of MinE changes. One β1-strand gets a helical conformation and binds the MinD dimer (and the membrane by its MTS). The other β1-strand does not become helical and binds the membrane without MinD binding. When MinD is released from the membrane by the MinE activity, MinE can (a) bind another MinCD complex at the membrane or (b) can release the membrane and return to its six-stranded conformation. *Park et al., 2011.*

interactions could cause the necessary conformational changes in the other MinD subunit and stimulate hydrolysis of the second ATP (Park *et al.*, 2012).

MinC

MinC is the actual division inhibitor in the Min system. It is the only Min protein that can inhibit the FtsZ polymerization and its presence at the cell poles prevents Z ring formation at that site (Rothfield *et al.*, 2005). It does not have topological specificity, but its specific distribution in the cell depends on the topological specificity factor MinE. The mapping of the MinE and MinC binding sites on the structure of MinD clearly shows that MinE and MinC have overlapping binding sites. During the oscillation cycle, MinE competes with MinC for MinD interaction at mid-cell and displaces it, thus reducing its division inhibiting activitiy (Rothfield *et al.*, 2005).

The way in which MinC inhibits the formation of Z rings is not entirely clear yet. For polymerization of FtsZ its GTPase activity is important. However, several studies have shown that MinC does not affect the GTPase activity of FtsZ, so it does not prevent Z ring formation by blocking the assembly of FtsZ molecules (Lutkenhaus, 2007). It was therefore suggested that MinC works via the stimulation of disassembly of FtsZ polymers and prevention of interactions between them.

Recent studies on the interactions between MinC and FtsZ showed results that support this idea and cleared up the mechanisms behind MinC action a bit more. It is known that MinC is a dimer and that each monomer consists of two domains of similar size that both have their own function in the inhibition of Z ring formation (Dajkovic *et al.*, 2008). The C-terminal domain of MinC ($MinC^C$: residue 116-231) is responsible for the dimerization of MinC and contains the region that binds MinD. Furthermore, it is thought to inhibit Z ring formation by disturbing the bundling of FtsZ polymers and displacing FtsA from the Z ring. The N-terminal domain of MinC ($MinC^N$: residue 1-115) interacts with FtsZ to weaken longitudinal bonds between the FtsZ molecules in the filaments. As a consequence, filaments break easier, resulting in shorter and curved polymers.

Protein structure of MinC

The crystal structure of MinC has already been determined in previous studies (Barák and Wilkinson, 2007) (Figure 8). The two domains of MinC are of similar size and can be easily distinguished in the structure. $MinC^C$ is the part that is involved in the dimerization of MinC and is connected to $MinC^N$ by a flexible linker (Barák and Wilkinson, 2007). The C-terminal domain of MinC consists of a triangular β-helix with one hydrophobic face involved in the dimer interactions. $MinC^C$ also contains the MinD binding region at the vertex of the triangular β-helix farthest away from the dimer interface. Therefore the two MinD binding sites in a MinD dimer are far apart and cannot interact with the same MinD dimer (Wu *et*

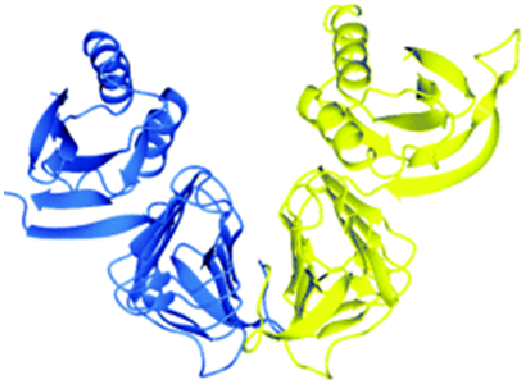

Figure 8: Structure of a MinC dimer. The monomers are colored blue and yellow. The C-terminal domain of each monomer is involved in the dimerization and is connected to the N-terminal domain by a flexible linker. *Barák and Wilkinson, 2007.*

al., 2011). The MinD binding region contains a highly conserved sequence, RGSQ, that has proven to be necessary for the interaction with MinD.

MinD-MinC interaction

In MinD, the binding site for MinC lies at the upper half of its dimer interface. Since this site is created by ATP-dependent dimerization, it seems likely that nucleotide binding and hydrolysis can affect the interaction between MinD and MinC (Barák and Wilkinson, 2007). Indeed, mutations that affect the ATP binding site on MinD, also affect the binding of MinC and MinE, indicating that ATP is required for correct MinC and MinE interactions (Zhou and Lutkenhaus, 2004).

Several studies have shown that MinC can inhibit cell division in the absence of MinD when it is overexpressed (Lutkenhaus, 2007). Nevertheless, MinD must have an important function, since binding to MinD results in about 25- to 50-fold higher inhibition activity of MinC, because it targets MinC to a component of the divisome. This targeting function of MinD was shown in GFP-fusion studies, in which a GFP-MinC mutant that could bind MinD but was unable to inhibit Z ring formation, was localized to the Z ring at mid-cell (Zhou and Lutkenhaus, 2004). Furthermore, through interaction with MinD, MinC inhibition activity can be spatially controlled by the topological specificity factor MinE (Lutkenhaus, 2007). It seems that these effects of MinD on MinC are caused by the binding of MinC to the membrane through the interaction with MinD. Indeed, MinC proteins that are fused to the MTS of MinD bind the membrane and show highly increased activity (Lutkenhaus, 2007).

It thus seems that the membrane-binding property of MinD plays an important role in the action of MinC. However, membrane binding of MinD is not required for MinC binding, since MinD mutants in which the MTS is truncated can still bind MinC (Zhou and Lutkenhaus, 2004). As expected, the MinD without MTS is unable to target MinC to components of the divisome, confirming that membrane-binding is required for the targeting function of MinD. It is suggested that FtsZ molecules are the target for MinC, because MinC interacts directly with FtsZ and does not show any interactions with other components of the Z ring (Dajkovic *et al.*, 2008; Shen and Lutkenhaus, 2009).

FtsZ-MinC interaction

As the effector of the Min system, MinC inhibits the formation of Z rings. It has been shown that the GTPase activity of FtsZ, that is involved in the formation of longitudinal interactions between the FtsZ molecules, is not affected by MinC. This suggests that MinC does not inhibit the assembly of FtsZ polymers, but works by another mechanism. Recent research has shown that MinC enhances the disassembly of the Z ring by weakening the longitudinal and lateral interactions between the FtsZ molecules, thus establishing that the assembly of the Z ring is not affected (Dajkovic *et al.*, 2008).

The two domains of MinC each have their own function in the Z ring disassembly. $MinC^N$ can inhibit cell division even in the absence of MinD when overexpressed and is able to disassemble FtsZ filaments *in vitro*. $MinC^C$ shows only inhibition activity in the presence of MinD and cannot disassemble the filaments on its own, although it weakens their lateral interactions. Nevertheless, fully efficient inhibition of cell division is only seen when full length MinC is present, showing that the domains work synergistically (Shen and Lutkenhaus, 2010). The two domains of MinC and their interactions with FtsZ will be discussed separately.

MinC^C interactions

The C-terminal domain of MinC has several important functions; it targets MinC to the Z rings to bring the N-terminal domain close to the FtsZ molecules and prevents lateral interactions between the FtsZ filaments. Moreover, recent studies have shown that it interrupts the interaction between FtsZ and FtsA, thus preventing the recruitment of downstream proteins that are necessary for cell division.

The targeting function of MinC^C has been elucidated in some recent studies. The C-terminal domain shows direct interaction with FtsZ, since sedimentation studies with FtsZ polymers and MinC^C show enrichment of the FtsZ pellet with MinC^C (Dajkovic *et al.*, 2008). Other direct interactions with Z ring components were not found, so it seemed that FtsZ is the target protein for MinC^C. FtsZ was confirmed to be the target for MinC by studies of Shen and Lutkenhaus (2009). After performing random mutagenesis on FtsZ, they screened for mutants that are unable to interact with MinD-MinC^C. The four mutants that were found are D373E, I374V, L378V and K380M. Especially FtsZ-I374V showed strong resistance to MinD-MinC^C. Interestingly, the mutated residues were all located in the extreme C-terminus of FtsZ, indicating that this C-terminus is very important in the interaction of MinC^C with FtsZ. Moreover, GFP-fusion studies showed that the interaction with the C-terminus is required for the targeting of MinC (Shen and Lutkenhaus, 2009). GFP-MinD-MinC^C is recruited to the Z ring when wild-type FtsZ is present in the cell, but when the FtsZ-I374V mutant is used, GFP-MinD-MinC^C is evenly distributed on the membrane. This showed that MinD-MinC^C is unable to bind to FtsZ-I374V and that the C-terminus of FtsZ is necessary for targeting.

Besides its targeting function, MinC^C also mediates directly in weakening the FtsZ polymers by reducing their lateral interactions. Dajkovic *et al.* (2008) confirmed the weakening effect that MinC^C has on the lateral interactions by EM studies on polymer networks. MinC^C clearly reduced the bundling of FtsZ polymers. They also performed rheometry studies and showed that the stiffness of FtsZ structures is reduced in the presence of MinC^C or MinC. Interestingly, in contrast ZapA, a protein involved in cell division, is known to promote the bundling of FtsZ filaments. Dajkovic *et al.* (2008) showed that ZapA can restore FtsZ polymer bundles and suggest that MinC^C and ZapA compete for binding sites on FtsZ because of their antagonistic activities on polymerization. However, they did not further investigate the interactions between these two proteins.

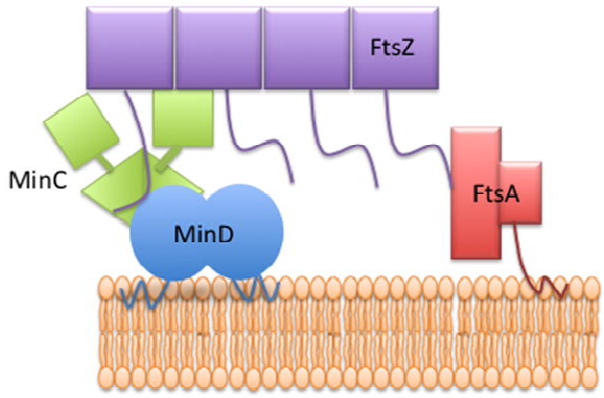

Figure 9: Overview of MinC action. MinC binds MinD by its C-terminal domain resulting in the targeting of MinC^N to the FtsZ polymers. There the N-terminal domain weakens the longitudinal interactions in the FtsZ polymers. MinC^C displaces FtsA from the FtsZ polymers by interaction with the C-terminus of FtsZ and weakens lateral interactions between the polymers. Because of the displacement of FtsA downstream division proteins cannot be recruited. *Wu et al., 2011.*

The C-terminus of FtsZ is highly conserved and interacts with many more proteins than just MinC. Two very important proteins in cell division that bind to the C-terminus of FtsZ are ZipA and FtsA. These proteins anchor FtsZ to the membrane where it can form a stable Z ring. When just one of these proteins is present, the Z ring can be formed, but the recruitment of downstream proteins fails and as a consequence the cell cannot divide (Shen and Lutkenhaus, 2009). According to yeast two-hybrid studies, ZipA can

still bind FtsZ when residue I374 in the FtsZ C-terminus is mutated, but FtsA cannot. This supports the idea that FtsA and MinC have overlapping binding sites at the C-terminus of FtsZ and that they might compete for binding (Shen and Lutkenhaus, 2009). This provides a possible mechanism for the inhibition action of $MinC^C$ in which MinC displaces FtsA from the FtsZ molecules in the Z ring, thus preventing the recruitment of required downstream division proteins (Figure 9). Shen and Lutkenhaus (2009) showed evidence for this mechanism in localization studies with GFP-MinD-$MinC^C$ and immuno-stained ZipA and FtsA. At low concentrations, GFP-MinD-$MinC^C$ is localized to ring structures and inhibits cell division. This indicates that it does not break down the Z ring, but is still able to stop cell division in another way. Localization of FtsA and ZipA at these low concentrations showed that there are far less FtsA rings than ZipA rings in the cell. At high concentrations, the GFP-MinD-$MinC^C$ was not localized to a ring structure anymore, suggesting that it broke down the Z ring. In support, at these high concentrations the rings of ZipA and FtsA all disappeared as well. Based on these results, Shen and Lutkenhaus suggest that MinD-$MinC^C$ displaces FtsA at low concentrations, thus preventing cell division but not breaking down the Z ring. At high concentrations, MinD-$MinC^C$ is also able to displace ZipA resulting in the breakdown of the Z ring. However, they provide no direct evidence that ZipA can be displaced by MinD-$MinC^C$ and that this displacement is the cause of Z ring breakdown.

$MinC^N$ interactions

The N-terminal domain of MinC alone (as monomer) shows the same division inhibition activity as full MinC *in vitro*, suggesting that this domain plays an important role in inhibition of cell division. $MinC^N$ has shown to weaken the longitudinal bonds between FtsZ molecules in filaments, thus reducing the stability of the polymer which subsequently breaks easier. EM studies on polymer structures of FtsZ confirmed this as they show that the filaments become shorter and curved when full $MinC^N$ is present (Dajkovic *et al.*, 2008).

The N-terminal domain can inhibit cell division without the presence of the C-terminal domain. A cell containing the FtsZ-I374V mutant is not inhibited for division by MinD-$MinC^C$ but is still sensitive to MinD-MinC (Shen and Lutkenhaus, 2009). This suggests that $MinC^N$ has its own binding site on FtsZ through which it performs its inhibition and that its actions do not depend on the C-terminus of FtsZ. In a screen for FtsZ mutants that do not respond to MinD-MinC Shen and Lutkenhaus (2010) identified two groups of mutants that are important for MinC action. The first group has mutations in the C-terminus of FtsZ and these mutations are thus thought to block the action of $MinC^C$. The second group of mutants that do not respond to MinD-MinC are therefore likely to be involved in the activity of the other domain, $MinC^N$. This group consisted of FtsZ-R271G, -E276D and -N280S. Remarkably, these three residues are located in an α-helix at the end of FtsZ, called helix H-10, that lies opposite the GTP-binding site. Thus, since three residues in this helix are critical for $MinC^N$ action, the binding site for $MinC^N$ seems to be in helix H-10 (Shen and Lutkenhaus, 2010).

The residue that is most critical for $MinC^N$ activity is N280, since mutation of this residue showed the highest resistance against MinD-MinC. Moreover, in a cell with the FtsZ-N280D mutant the Min system activity is completely suppressed. Nevertheless, this FtsZ mutant is still sensitive to $MinC^C$, confirming that $MinC^C$ and $MinC^N$ have different binding sites and that they inhibit division by different mechanisms. Furthermore, sedimentation tests revealed that FtsZ-N280D cannot bind $MinC^N$, although wild-type FtsZ is able to do this, thus supporting the idea that helix H-10 functions as binding site for $MinC^N$ and that N280 is critical in the interaction.

However, the exact way in which $MinC^N$ weakens longitudinal bonds in FtsZ by binding to the H-10 helix is not entirely clear. Shen and Lutkenhaus (2009) tried to eludicate this mechanism by looking at the binding site of $MinC^N$ on FtsZ. The H-10 helix lies close to the interface between FtsZ subunits in a filament. The GTPase activity of FtsZ takes place at this interface as well and might thus be involved in $MinC^N$ action. With a NADH-coupled enzymatic assay Shen and Lutkenhaus (2009) showed that FtsZ-N280D has a reduced GTPase activity (~60% of wild-type activity). In addition, the FtsZ mutant shows slightly less polymerization. The N280 residue thus seems to be involved in GTPase activity as well as $MinC^N$ activity. This is not surprising, because both actions occur near the H-10 helix at the interface of FtsZ molecules. Based on these results, Shen and Lutkenhaus (2010) proposed a model for MinC action in which GTPase activity is linked to the action of $MinC^N$. They suggest that GTPase activity induces small conformational changes in the FtsZ-FtsZ interface that affect the binding of $MinC^N$. The FtsZ-subunits in a filament have a more open conformation and are less strongly attached to each other when they are bound to GDP compared to GTP, making the H-10 helix more accessible for interaction with $MinC^N$. Therefore, $MinC^N$ would be able to bind GDP-FtsZ subunits and weaken their longitudinal interactions with other subunits. This would result in shorter polymers and inhibition of Z ring formation. However, there is no convincing evidence that $MinC^N$ rather binds GDP-FtsZ than GTP-FtsZ, so this model seems not very strong.

Sensitivity of MinCD

A recent study of Shen and Lutkenhaus (2011) revealed that the MinCD complex has different sensitivity to internal and polar Z rings. They found this remarkable characteristic of MinCD while studying the minicelling phenotype of FtsZ-I374V. However, further research established that other strains, including wild-type FtsZ strain, show this difference in sensitivity as well.

Examination of the cell morphology of the FtsZ-I374V mutant revealed that many minicells are formed in the absence of MinCD and MinE (Shen and Lutkenhaus, 2011). This is not surprising, since the Min system is not present to inhibit cell division over the cell poles. Subsequently, Shen and Lutkenhaus raised the concentration of MinCD. Remarkably, at a certain concentration of MinCD they observed that less minicells were formed but that the cells still show proper division at mid-cell. This suggests that MinCD at this concentration only blocks the polar divisions and not the internal divisions, though MinE is absent. When the concentration was raised even further, the cells became filamentous and died, indicating that at relatively high concentrations MinCD blocks internal divisions as well. Thus, assuming that MinCD is evenly distributed in the cell in absence of MinE, MinCD seems to show a different sensitivity to polar and internal divisions.

The way in which MinCD would achieve this difference in sensitivity to polar and internal division sites is not yet understood. Since MinCD is evenly distributed on the cytoplasmic membrane, an increased amount of MinCD at the poles cannot be the cause of the specific polar inhibition. One option is that there is a difference between polar and internal Z rings or that internal Z rings are better protected near mid-cell by other topological specific factors than MinE. Another possibility is that special polarly localized factors are able to increase the activity of MinCD at the poles. However, such factors have not yet been found (Shen and Lutkenhaus, 2011).

The requirement of MinD for the different sensitivity of MinCD was investigated as well. Shen and Lutkenhaus (2011) show that MinC fused to the MTS from MinD also has different sensitivity for polar and internal Z rings. MinC without MTS does not show the

sensitivity differences, thus indicating that MinD is not required for the sensitivity, although binding of MinC to the membrane is crucial.

In previous models of the Min system it was always assumed that MinCD has the same sensitivity for FtsZ throughout the cell. If further research confirms the existence of different sensitivity of MinCD this would raise a lot of new questions, for instance about the role of MinE in the system (Shen and Lutkenhaus, 2011). The topological specificity of MinE seems less necessary if MinCD shows its own topological specificity through the difference in sensitivity.

The Min system in other bacteria

It is important to mention that the Min system as described above is not present in all rod-shaped bacteria, although the Min proteins are widely conserved (Lutkenhaus, 2007). For instance, Gram-positive bacteria, like *Bacillus subtilis,* contain MinD and MinC homologues, but no MinE homologue (Rothfield *et al.*, 2005). Moreover, oscillation of Min proteins is not observed in *B. subtilis* and two additional proteins, DivIVA and MinJ, seem to substitute the topological specificity function of MinE. DivIVA is localized at cell poles and stabilizes MinCD positioning over there, thus creating a low MinCD concentration zone at mid-cell. DivIVA does not directly bind MinCD, but needs MinJ to mediate in this interaction (Pavlendová *et al*, 2009). There are also bacteria that do not seem to contain any proteins like those in the Min system, e.g. *Caulobacter crescentus*. These bacteria must regulate the precise positioning of the divisome by other mechanisms, including nucleoid occlusion (Rothfield *et al.*, 2005).

Concluding thoughts and future perspectives

Many mechanisms in cell division are still not fully unraveled and give many possibilities for further research. One intriguing action of the bacterial cell in cell division is the precise positioning of the Z ring at mid-cell. Although the Min system in *E. coli* has been extensively studied compared to other division regulating mechanisms in bacteria, this system still carries a lot of secrets. Nevertheless, recent findings shed a new light on some parts of the system, giving us the opportunity to unravel its molecular mechanisms and propose new and more detailed models for Min protein actions.

One of the most striking findings of the past years is the different sensitivity of the MinCD complex for polar and internal Z rings. The idea that MinCD preferentially inhibits polar division sites was never considered in the existing models and would raise a lot of new questions when its correctness is proved, for instance about the usefulness of topological specificity factor MinE or about the existence of specific polarly localized factors that interact with MinCD. Nevertheless, the sensitivity phenomenon must be further investigated before any strong conclusions can be drawn.

Because of the development of methods for protein structure determination, more and more protein structures can be solved at high resolution, giving us the opportunity to study dimerization interactions, conformational changes and binding sites of molecules. However, because the proteins of the Min system are co-operating in a complex, their individual stability is most times low, making it hard to obtain useful crystals.

Furthermore, the Min proteins have a strong tendency to oligomerize and aggregate. The crystal and NMR structures of Min proteins are many times obtained from mutated Min proteins or orthologues from other organisms than *E. coli* to overcome these problems (Barák and Wilkinson, 2007). Nevertheless, these non-*E. coli* and non-wild-type structures have proven to be very useful by elucidating interaction mechanisms between the *E. coli* Min proteins.

The protein structures gave a lot of new information on the interactions of Min proteins and many residues have been identified that are involved in these interactions. The next step is to use this information to unravel the exact molecular mechanisms in the proteins. This has already been tried for the ATPase activity of MinD. Although this activity is present in many proteins and the requirements for efficient hydrolysis are already known, it appears to be very difficult to determine the molecular mechanism for this action in MinD.

Thus, there still are plenty possibilities for future research on the *E. coli* Min system. Recent findings refined and cleared existing models of the positioning of the Z ring at mid-cell, but exposed previously unknown actions of the Min proteins as well, thus raising many new questions about the system. By thoroughly comparing the Min system with analogue systems in other bacteria that also regulate proper positioning of the division site one might also get a better understanding of its actions.

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