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Assembly Dynamics of the Bacterial MinCDE System and Spatial Regulation of the Z Ring

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Abstract

The positioning of a cytoskeletal element that dictates the division plane is a fundamental problem in biology. The assembly and positioning of this cytoskeletal element has to be coordinated with DNA segregation and cell growth to ensure that equal-sized progeny cells are produced, each with a copy of the chromosome. In most prokaryotes, cytokinesis involves positioning a Z ring assembled from FtsZ, the ancestral homologue of tubulin. The position of the Z ring is determined by a gradient of negative regulators of Z-ring assembly. In *Escherichia coli*, the Min system consists of three proteins that cooperate to position the Z ring through a fascinating oscillation, which inhibits the formation of the Z ring away from midcell. Additional gradients of negative regulators of FtsZ assembly are used by *E. coli* and other bacteria to achieve spatial control of Z-ring assembly.

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Cytoskeletal: protein filaments that provide for intracellular organization

INTRODUCTION

Most bacteria grow and divide by a process referred to as binary fission; a process in which a cell increases in size and splits into two nearly equal daughter cells. Growth is accompanied by replication and segregation of the chromosome with each daughter cell receiving a copy at division. This simple cell cycle was revealed through the use of a light microscope. Closer examination of dividing cells with an electron microscope revealed a septum formed at midcell by the invagination of the cytoplasmic membrane and ingrowth of the cell wall. Various studies revealed that ingrowth of the cell wall involved the localized synthesis of peptidoglycan at the septum. However, electron microscopy did not reveal any cytoskeletal elements as observed in eukaryotic cells associated with the invaginating envelope, leaving the bacteriologist to wonder how this symmetrical invagination of the cell envelope was orchestrated. Measurement of the position of the septum in rod-shaped cells revealed that it was positioned fairly precisely at midcell (1). How was this spatial regulation achieved? What was being regulated?

This simple description of bacterial cell division was the extent of our knowledge until the 1990s when the application of genetics and cell biology techniques revealed many of the genes required for cell division. Furthermore, these studies revealed that all essential division proteins are localized to the septum and that localization is crucial for their function. Critical among these is FtsZ, the bacterial homologue of tubulin, which assembles into a cytoskeletal element, the Z ring, which recruits at least a dozen other proteins to form a complete septal ring able to carry out cytokinesis (2–4). Subsequent studies demonstrated that the position of the Z ring dictates the position of the septum (5, 6) and that the Z ring is positioned precisely at midcell (7). Thus, the question became how is the Z ring positioned to midcell? It also emerged that a Z ring could form anywhere in the cell as opposed to earlier thought that specific sites existed at midcell and the pole (7). Therefore, the question could be rephrased, "How is assembly of the Z ring limited to midcell?"

THE CYTOKINESIS MACHINERY

A genetic approach to studying cell division in *Escherichia coli* led to the identification of a dozen genes that are essential to this process (3, 4). Most of these genes carry the *fts* designation because of the filamenting temperature-sensitive phenotype displayed by strains carrying conditional lethal mutations in the corresponding genes. FtsZ was localized to the division site by immunoelectron microscopy, and subsequent application of fluorescence microscopy revealed that the other *fts* gene products are also localized to the division site (2, 8). Studies to determine the order of addition of the gene products to the division site indicated that the order is hierarchical, with a downstream gene product depending upon the localization of all upstream division proteins (9). Nonetheless, some of the later gene products appear to exist in a complex outside of the division site (10). It is possible that each gene product interacts with multiple partners for stable localization at the septum.

Another aspect of the localization of these division proteins is the temporal pattern of recruitment during the cell cycle. This pattern separates the division proteins into two distinct groups, those arriving early to establish the Z ring and those arriving about 10 min later (~1/2 of the cell cycle at rapid growth rate) to form the complete septal ring capable of carrying out cytokinesis (11). For example, in rapidly growing *E. coli*, about 90% of cells contain the first group localized to the division site, but only about 40% to 50% of the cells have the second group localized. The first group includes FtsZ, FtsA, and ZipA, which arrive simultaneously at the division site and show codependency (12–14). Another protein, ZapA, also arrives at this time but is not essential (15). The second group includes FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI, and FtsN, which arrive after a distinct lag (11). The localization of this latter group depends upon the first group and arrives more or less simultaneously to form the complete septal ring capable of carrying out division. Additional proteins, AmiC and EnvC, although not essential, can be included in this latter group because they contribute to the efficiency of cell separation (16, 17). This review is concerned with the first group of proteins as they assemble into the Z ring that defines the division plane and is the point at which cytokinesis is regulated.

Z ring

FtsZ has low sequence identity (~10%) to tubulin, too low to be considered a homologue on this basis alone (18, 19). However, FtsZ has many of the properties of tubulin, and the structures of the two proteins are so similar that there is little doubt that the two proteins are homologues (20, 21). Nonetheless, it is surprising that these structural proteins share such limited sequence identity, whereas many enzyme homologues share a much higher level of sequence identity across the evolutionary divide. A more detailed analysis of the structural biology of FtsZ and other bacterial cytoskeletal proteins can be found in a recent review in this series (21).

FtsZ has been studied from two perspectives: localization in vivo and its ability to assemble in vitro. FtsZ, like tubulin, undergoes GTP-dependent assembly in vitro and displays a critical concentration for assembly around 1 μM. FtsZ displays very little lag in assembly and reaches steady state in less than 30 s (22–25). This lack of a kinetic barrier to assembly suggests that FtsZ assembly, unlike that of tubulin, is not regulated at the nucleation step. For both tubulin and FtsZ, the basic assembled unit is the protofilament, a head-tail linear arrangement of subunits (26). In the case of tubulin, isolated protofilaments are not observed but are associated laterally to produce a microtubule. With FtsZ, lateral associations are much weaker, and a well-defined assembled structure beyond the protofilament has not been observed, although a variety of bundled

FtsZ: the bacterial homologue of eukaryotic tubulin

Z ring: a cytoskeletal element in bacteria that is the scaffold for the septal ring

Septal ring: a structure containing all of the proteins required for bacterial cytokinesis

Cytokinesis: the division of a cell into two

Protofilament: a polymer consisting of a linear array of assembled monomers

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structures are formed depending upon the in vitro conditions.

FRAP: fluorescence recovery after photobleaching

Min: minicell phenotype

An important feature of FtsZ assembly in vitro is the high GTP hydrolysis rate (10 per min) that is coupled to assembly (22, 24). This rapid rate indicates that subunits are entering and exiting a protofilament with a half-life on the order of 10 s. The implication of this high rate of turnover is that FtsZ protofilaments can only achieve an average length of 30 subunits or about 0.1 μm as longer filaments could not achieve the high turnover rate (24). Because the circumference of the division site in bacteria is ∼3 μm, this would mean that the Z ring consists of a network of short protofilaments.

FtsZ was first shown to be localized to the division site by immunoelectron microscopy (2). These studies indicated that FtsZ was in a ring-like assembly, which was subsequently referred to as the Z ring (27). Additional studies, using fluorescence microscopy, confirmed the ring structure (28). The arrangement of FtsZ polymers in the ring has not yet been detected by electron microscopy, and the existence of a structure is inferred from FtsZ's ability to assemble into polymers in vitro. An important unanswered question is the structure of the Z ring.

The ability to monitor cells, expressing a fraction of their total FtsZ as FtsZ-GFP (green fluorescent protein), has allowed exploration of the dynamics of the Z ring. FtsZ-GFP is not fully functional in *E. coli*; however, expression of the fusion at <20% of the total FtsZ does not appear to interfere with division. Fluorescence recovery after photobleaching (FRAP) demonstrated that FtsZ in the ring is highly dynamic, which is consistent with the rapid assembly and disassembly observed following temperature shifts with the FtsZ84 temperature-sensitive mutant (29–31). The FRAP studies indicated that FtsZ in the ring has a half-life on the order of 10 s. This is remarkably similar to the GTPase rate observed in vitro, suggesting that the flux of FtsZ in and out of the ring may be dictated by the intrinsic GTPase in vivo.

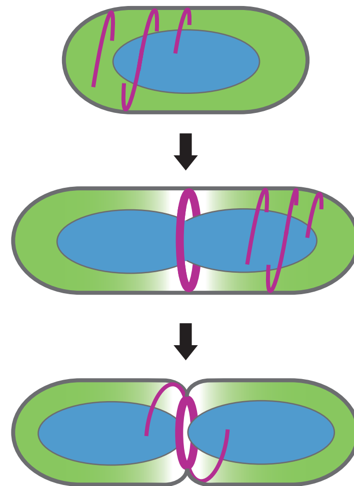

Figure 1

Z ring. The Z ring assembles at midcell where the concentration of negative regulators of assembly is lowest. FtsZ is also observed in rapidly migrating helices. These helices may be membrane-bound FtsZ polymers that are precursors to the ring (32, 33).

Another feature of FtsZ dynamics revealed by utilizing FtsZ-GFP is the helix-like waves of FtsZ that move rapidly throughout the cell along the membrane (32). These waves can be visualized in small cells that lack a ring but are also observed in cells with a ring (**Figure 1**). The movement of these waves is not dependent upon the Min system, but their orderly migration appears to depend upon Min. The link between these waves and the ring is not entirely clear. It is likely that they are polymers attached to the membrane and are precursors and/or turnover products of the ring. As mentioned above, FtsZ has a low critical concentration (∼1 μM compared to an estimated in vivo concentration of 5–10 μM) and only displays a small kinetic lag to assembly, so these helices may represent FtsZ's constant state of assembly/disassembly. Z-ring formation likely results from a coalescence of these membrane-bound helices, where the influence of negative regulators is lowest (33).

The *ftsZ* gene is a very highly conserved cell division gene and is present in most

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bacteria and Archaea (34). Thus, the Z ring is likely to be the cytoskeletal element that drives cytokinesis in most prokaryotes. FtsZ is also present in chloroplasts and in mitochondria of some algae and amoeba but not in those of higher plants or animals. However, some bacteria do not contain FtsZ, and it will be of interest to determine the cytoskeletal element that drives cytokinesis in these organisms.

Tethering FTSZ to the Membrane

The formation of the Z ring requires the presence of a protein that can tether FtsZ filaments to the membrane. In *E. coli*, both ZipA and FtsA can perform this function (12). These proteins bind to a short conserved region near the extreme C-terminal tail of FtsZ as well as to the membrane (35, 36). Z rings are present in filamentous cells depleted for one of these proteins but not if both are depleted. For example, an *ftsA* (Ts) mutant does not divide at the nonpermissive temperature, but Z rings are present throughout the filament at expected division sites due to ZipA (12). Also, FtsZ, with an altered C-terminal tail that is unable to bind these two proteins, fails to assemble into a Z ring. In these instances, the FtsZ appears to accumulate in disordered structures between nucleoids.

ZipA binds to the membrane through an N-terminal transmembrane segment (13). This segment is more than a simple membrane-anchoring domain because it can not be replaced by transmembrane segments of another membrane protein. FtsA, which is related to actin (37), contains a C-terminal sequence that can function as a membrane-targeting sequence (MTS) through formation of an amphipathic helix (33). FtsA's MTS does not appear to be specific as it can be replaced by the MTS from MinD.

The ratio of FtsZ to ZipA or FtsA is critical for cell division (13, 38). Depletion of any of these proteins blocks cell division, but overproduction does as well. As little as a twofold increase in ZipA leads to filamentation, whereas with FtsA, cell division is blocked if FtsA is overproduced about 5- to10-fold (13, 38, 39). In both cases, the block can be relieved if FtsZ is also increased. Therefore, cell division not only requires a minimum amount of these proteins but also that they be maintained within a certain ratio. The block to division appears to result from a disturbance in Z-ring structure. As the level of any one of the proteins is increased, the Z ring appears to dissipate into spirals that are spread along the length of the cell (28, 33). These results indicate that the proper ratio of FtsZ to its membrane-anchoring partners is required for assembly and maintenance of the Z ring. In contrast, cell division is relatively insensitive to increases in levels of downstream division proteins.

MinD: a membrane ATPase, which activates MinC, stimulated by MinE

Although either FtsA or ZipA is sufficient to promote Z-ring assembly, both are required for recruitment of downstream proteins (12). Thus, Z rings formed in the presence of FtsA, but lacking ZipA, lack all downstream proteins (12, 40). Even though both FtsA and ZipA are required for downstream recruitment, the role of FtsA is thought to be more direct for two reasons. First of all, a unique mutation in *ftsA* bypasses the requirement of ZipA, arguing that ZipA may not be directly involved (39). The *ftsA* bypass mutation is interesting because it appears to stabilize the Z ring as it is more resistant to the destabilizing effects of the inhibitor MinCD (see below). Second, interaction between FtsA and other proteins has been observed in two-hybrid systems (41, 42). Such studies indicate that FtsA interacts with the downstream proteins FtsI and FtsN.

Both FtsA and ZipA are able to link FtsZ to the membrane, but they are not conserved in all organisms. Of the two, FtsA is more widely conserved, although it is not present in actinomycetes, cyanobacteria, mycoplasmas, chloroplasts, or Archaea. ZipA is only found in bacteria closely related to *E. coli*, although some gram-positive bacteria have a topologically similar protein, EzrA, that may play a similar role (13, 43). Some bacteria and chloroplasts that lack ZipA and FtsA have a

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MinC: an inhibitor of FtsZ assembly

MinE: a topological regulator of MinCD

protein with a DnaJ domain linked to a transmembrane domain (44). Such a protein could function to tether FtsZ filaments to the membrane. It is not clear if these proteins play a role in addition to membrane tethering. FtsA has been shown to self-interact and even polymerize (45, 46). ZipA has been found to self-interact in a bacterial two-hybrid screen (47). Such self-association could provide a cross-linking function for an FtsZ network. ZipA has also been shown to bundle FtsZ filaments under some conditions (48, 49).

A BRIEF HISTORY OF THE MIN PHENOTYPE

Investigation into the genetics of the spatial control of cell division in *E. coli* started with the isolation of the *min* mutant by Adler et al. (50). This mutant was isolated on the basis of resistance to ionizing radiation while retaining sensitivity to UV light. The classic phenotype of this mutant is the production of small anucleate minicells from the poles of rod-shaped mother cells, which occurs under a variety of growth conditions. Later studies confirmed that minicells were produced by the same division machinery utilized to divide cells at midcell (51). Minicell formation is not unique to *E. coli* and has been reported in a variety of rod-shaped bacteria, either owing to growth conditions or mutation (52).

Importantly, a consequence of minicell production is an increased average cell length of the DNA-containing mother cells. A quantitative examination of the cell length distribution led to the proposal that a quantum of "division factor" is produced each cell doubling (53). In this model, the poles are available for division and compete for the midcell site for a limited amount of division factor. As a consequence, a division event at a cell pole occurs at the expense of the midcell division, resulting in a longer mother cell. As part of this model, it was assumed that the function of Min is to inactivate the poles so that they are no longer available to the division factor. Significantly, increasing FtsZ levels in the *min* mutant reduces the average cell size, which led to the suggestion that FtsZ is the limiting division factor (54).

An early result, indicating antagonism between the Min system and FtsZ, was the observation that overproduction of FtsZ caused minicell formation in an otherwise wild-type strain (51). This result is consistent with the function of Min in preventing polar divisions and indicated that excess FtsZ can overcome Min inhibition at the poles to produce minicells. Consistent with this, increased FtsZ suppressed the inhibition of division caused by overexpression of the MinCD inhibitor (55). Also, mutations in *ftsZ* that reduce the GTPase activity and result in less dynamic polymers display increased resistance to MinCD (56, 57).

A more recent result also highlights the interplay between Min and FtsZ and demonstrates an important point. A Δ*min* mutation is incompatible with the *ftsZ84* (Ts) mutation above room temperature (58). The FtsZ84 protein has reduced activity, and its partitioning among several Z structures at the poles and between nucleoids in the *min* mutant is thought to prevent any of them from maturing into a functional Z ring. This notion that partitioning of FtsZ among too many ring structures can lead to inhibition of cell division is observed in other situations as well (see below).

The elaboration of the mechanism of Min action started with the cloning of the *min* locus in *E. coli* (59). This analysis also revealed that *min* was nonessential, and complete loss of Min function leads to the minicell phenotype. Sequence analysis revealed an operon containing three loci designated *minC*, *minD*, and *minE*. Differential expression of these genes in a strain deleted for *min* demonstrated that MinC and MinD cooperated to form a topologically nonspecific inhibitor of division that was restricted to act at the poles by MinE. Thus, MinE was designated the topological specificity factor. Subsequent studies revealed that MinC alone could inhibit division if overproduced 25- to 50-fold and that the function

of MinD was to enhance MinC's activity and mediate regulation by MinE (60). Interestingly, the *min* operon complements a *min* deletion mutant if present on a low-copy vector but not on a high-copy vector, indicating the level of the Min proteins has to be within a certain range (from ~1–10 times the physiological level) to be functional (61). Numerous studies have revealed that MinD is at the center of the system, interacting with both MinC and MinE.

A REMARKABLE OSCILLATION

The fascinating aspect of the Min system is its ability to inhibit division at the poles while allowing it to occur at midcell. How is this achieved? Initial models were static and involved MinCD localized at the poles and MinE localized at midcell where it acted as a shield to prevent MinCD action (62, 63). Such models were supported by an initial report of MinE localized near midcell independent of FtsZ (64). However, no one anticipated the remarkably dynamic behavior of the system, which was only revealed by the application of GFP-fusion technology. A groundbreaking report demonstrated that MinD undergoes a remarkable oscillation with a periodicity on the order of ~40 s (at 20°C) (65). During an oscillation, MinD forms a polar zone on the membrane, extending toward midcell, which then starts to shrink toward the pole. As this polar zone disappears, a new polar zone is established at the other end, and the cycle is repeated (**Figure 2**).

The oscillation of MinD is dependent upon MinE (65). In the absence of MinE, MinD is located along the cell periphery, indicating it is bound to the membrane. Importantly, the frequency of the oscillation is dictated by the MinD to MinE ratio. Increasing the ratio severalfold slows the oscillation, and minicells start to form. Subsequent studies revealed that MinE also oscillated in a pattern that indicated it formed a ring at the edge of the MinD polar zone (66, 67); an inference nicely confirmed by dual labeling with GFP variants (68). Although most MinE is present as a ring at the edge of the MinD polar zone, some MinE is present throughout the MinD polar zone. MinC was subsequently found to oscillate in a pattern that matched that of MinD but played no role in the oscillation (69, 70). Thus, MinC, the effector (division inhibitor) of the Min system, is a passenger in the oscillation.

One of the intriguing aspects of Min oscillation is how MinD released from one pole starts to assemble at the other pole. Does the pole play a special role, perhaps nucleating MinD assembly? The early results indicated this was unlikely because MinD oscillated in a striped pattern in cells induced to filament (65). When cell division is inhibited and cells start to elongate, the oscillation switches from a pole-to-pole pattern to pole to midcell. In even longer cells, the pattern becomes striped with the distance between maxima in the oscillation about 7–8 μm. This pattern ruled out the pole as playing a critical role, although it still left open the possibility that some marker is positioned along the cell length during growth that plays the same role as the pole.

Oscillation of Min proteins is also observed in round cells. Round *E. coli* can be generated by deleting genes, *rodA*, *pbpA*, or *mreB*, required for the maintenance of rod shape (71, 72). Division of round *E. coli* often generates cells that are kidney shaped, so that a long axis still exists. In these instances, the Min proteins oscillate on the long axis (73). However, in cells that appear symmetrical, a regular oscillation is still observed in the majority of cells (74). This oscillation may be due to a slight asymmetry in cell shape, which is difficult to determine in round cells. The observation that Min oscillation is observed in round cells and in striped patterns in filaments suggests the system does not require positional markers but is only affected by cell length and shape.

More recent examination of the Min oscillation, involving deconvolution of the fluorescent images, revealed that the oscillation of

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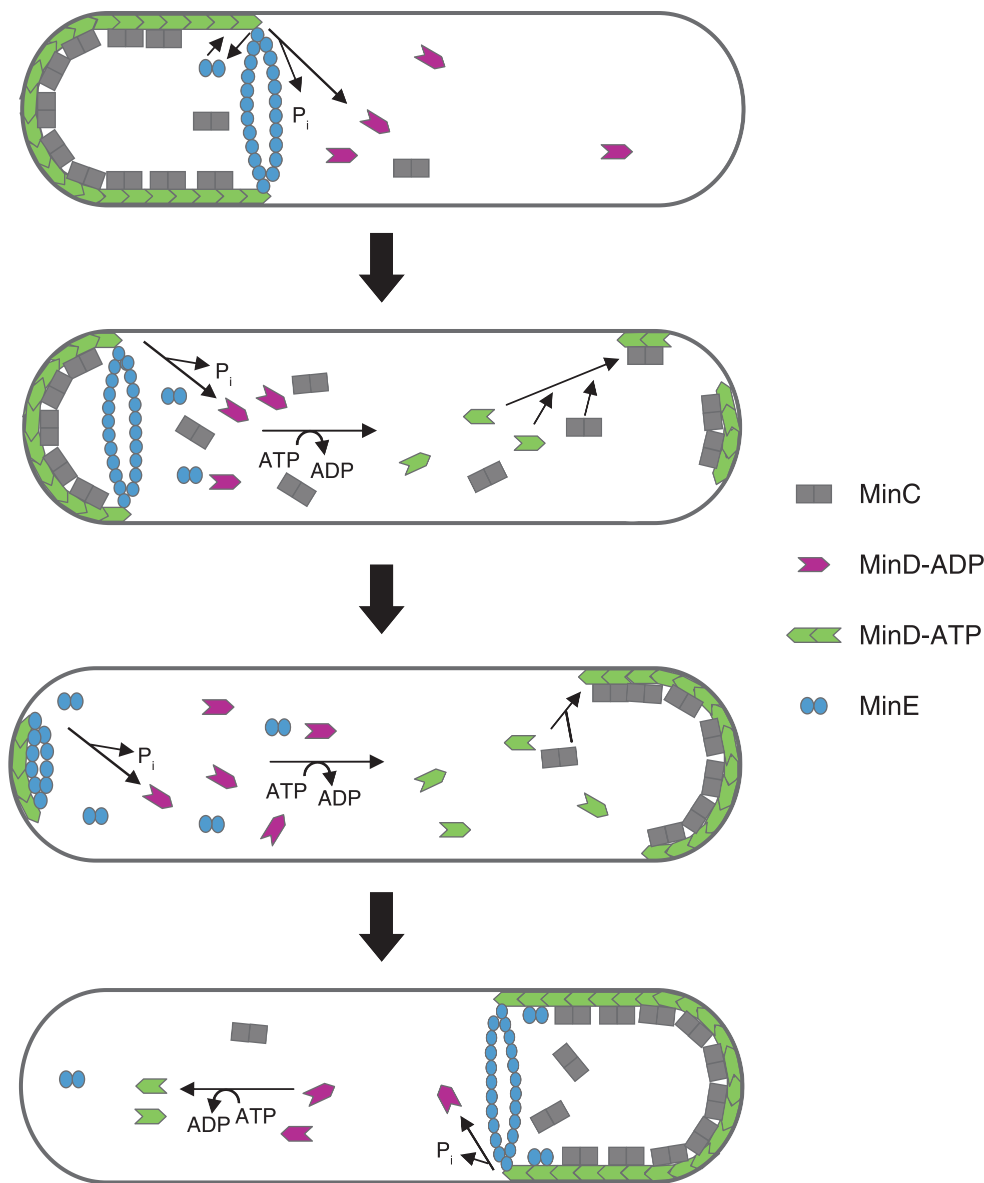

Figure 2

Oscillation of the Min proteins in *E. coli*. MinD-ATP binds to the membrane and recruits MinC. MinE displaces MinC and stimulates MinD ATPase, causing release of the proteins from the membrane. Whereas released MinE can immediately rebind to MinD on the membrane, the released MinD must undergo nucleotide exchange to regenerate MinD-ATP. In the model by Huang et al. (135), the concentration of MinD-ATP in the vicinity of the old pole is lowered because it binds cooperatively to the membrane already containing bound MinD. In contrast, the MinD-ATP concentration increases at the other pole, which lacks bound MinD. As the concentration rises, it eventually binds, forming a new polar zone. As MinE is released from the old pole, it binds to the ends of the MinD polar zone (135).

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MinD involved membrane-associated coiled structures (75). Although hinted at in unfixed images, more detail was obtained from optical sectioning and deconvolution of fixed cells. This study revealed that MinE and MinC were also present in coiled arrays that were entirely dependent upon MinD. MinE accumulated at the edge of the MinD coiled arrays near midcell, forming the MinE ring. Interestingly, this study found that faint MinD coils were present throughout the cell. This study mostly analyzed fixed cells, which appear to have less dramatic distributions of the Min proteins than living cells. Whereas living cells show very dramatic asymmetric distribution of the Min proteins, fixed cells are much less dramatic, raising a concern that the process of fixation may cause some redistribution. As mentioned above, the oscillation is observed in Δ*mreB* cells, indicating that the actin-like cytoskeleton due to MreB is not the basis for the MinD coiled array (74).

Since the reported oscillation of MinD, several other members of the ParA family (see below) have been observed to undergo oscillation. Soj (chromosomal ParA homologue), SopA (F plasmid), and ParA (plasmid pB171) are involved in DNA segregation, although Soj has an additional role in regulation of entry into sporulation (76). These proteins oscillate on or between nucleoids (77–79). For oscillation, both a partner protein (SpoOJ, SopB, and ParB, respectively) and the site to which it binds are required. Although oscillation is observed with these proteins, the periodicity is slower and more erratic. Nonetheless, it suggests that this family of proteins is suited to dynamic pattern formation.

THE MIN SYSTEM AND SELF-ORGANIZATION

The discovery of the Min oscillation is a significant example of self-organization in bacteria. Self-organization refers to the appearance of ordered behavior or structures owing to interactions of the system's components. This ordered behavior is not anticipated from the knowledge of the components but arises through their interaction within a defined parameter space. A key feature of self-organization is that energy consumption, usually nucleotide hydrolysis, allows for dynamic behavior, in contrast to self-assembly where there is no energy flow (80). The dynamics expand the repertoire of behavior that a system can exhibit, and the system can establish a pattern from random fluctuations in a homogeneous distribution without other cues. Thus, after division, a pattern can be reestablished in newborn cells. The relative simplicity of the Min oscillation, with only a few components known to be required (MinD, MinE, ATP, and an enclosed vessel), make it an attractive system to try and understand the basis for the oscillation. Thus, investigation into the behavior of the Min system has occurred at two levels. One is an investigation into the biochemistry of the proteins, and the second is computational modeling to simulate the process to ascertain the key features of the system.

INTERACTION AMONG MIN PROTEINS AND THE MEMBRANE BASIS FOR THE OSCILLATION

The three Min proteins have been intensely studied. Structures of all three proteins from *E. coli* or another organism have been obtained. Although the Min proteins are widely conserved, they are not present in all organisms. However, the genes are present in bacteria that branch close to the root of the phylogenetic tree of life (*Thermatoga maritima* and *Aquifex aeolicus*), suggesting that they were present in ancient bacteria but were subsequently lost in some lineages (81–83). There are also variations. *Bacillus subtilis* lacks MinE but contains an unrelated protein DivIVA that is responsible for spatial regulation of MinCD (84). Chloroplasts contain MinDE, but no MinC homologue has been indentified (85). Many Archaea contain two MinDs, one with and one without a membrane-binding domain

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(83). No MinC or MinE have been found in these organisms.

WACA: Walker A cytoskeletal ATPase

MinD

The MinD protein is an ATPase that is a member of a large family of ATPases,which includes ParA proteins involved in plasmid segregation (86–88). Additional members include the Soj family, which are found on bacterial chromosomes and have a role in chromosome segregation and in coupling chromosome segregation to development in bacteria, such as *Caulobacter crescentus* and *B. subtilis* (89–93). It should be noted that the nomenclature is confusing because ParA is used to refer to plasmid and chromosomal proteins even though they are distinct (94). Also, the plasmid ParAs form a very diverse subgroup with varying degrees of homology (95). A recent addition to this group is MipZ, found in *C. crescentus* and other members of the α-proteobacteria, which are involved in spatial regulation of Z-ring assembly (96). More recently this family of proteins has been referred to as WACA (Walker A cytoskeletal ATPase) as several members have been shown to assemble into polymers (21). More distant members include NifH, the Fe protein in the nitrogenase complex, and ArsA, a transporter involved in efflux of certain anions (97, 98).

The structures of several WACA proteins as well as two of the more distant relatives have been solved and display remarkable similarities despite limited identity in the primary sequences. A unique feature these proteins share is a variation on the Walker A motif, termed the deviant Walker A motif (KxxxxGKT), which includes two lysines (87, 88). In the most studied example, NifH, the N-terminal (signature) lysine reaches across the dimer interface to contact ATP bound to the other subunit, indicating dimerization, and this lysine contact is required for ATPase activity (note NifH is always a dimer because of the bound iron but becomes a more compact dimer that includes the lysine contact upon ATP binding) (97). Recently, a catalytically deficient mutant of Soj was crystallized as a dimer in the presence of ATP (99). The signature lysine displayed similar behavior to that observed with NifH. Furthermore, the Soj dimer was superimposable on the NifH dimer. So far, MinD proteins have been crystallized from Archaea species and are monomers even in the presence of a nonhydrolyzable analogue of ATP (100–102). However, MinD is not activated (to bind the membrane) by the ATP analogue used in the crystallization (103). MinD has been observed to undergo ATP-dependent dimerization in vitro, indicating it is also a dimer (104).

MinD Binding the Membrane

A unique feature of MinD among the WACA family members is the presence of an MTS at the C-terminal end of the protein (104, 105). The MTS is present in MinDs from bacteria, Archaea, and chloroplasts and is responsible for anchoring MinD to the membrane. The MTS displays remarkable similarity to the MTS found at the N terminus of Arf proteins involved in vesicle trafficking in eukaryotic cells (106). The MTS of *E. coli* MinD binds preferentially to anionic phospholipds and several of the large hydrophobic residues within the helix insert into the bilayer (107, 108). The MTS of gram-positive bacteria is three amino acids longer than the MTS of gram-negative bacteria, indicating a possible higher affinity for the membrane. The binding of MinD from *E. coli* to the membrane is ATP dependent (103). The ATP dependency is due to the necessity for MinD to oligomerize to lock it on the membrane (104, 109). In contrast, MinD from gram-positive bacteria may not have to oligomerize to bind the membrane indicating it may be ATP-independent.

The MTS of the *E. coli* MinD is insufficient to promote membrane binding of GFP in vivo unless it is repeated twice in tandem (109). This result is consistent with the proposal that MinD bound to the membrane

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is a dimer. MinD dimerizes in vitro in the absence of membrane, suggesting that it is similar to Soj, which forms a dimer in the presence of ATP (99, 110). Does MinD dimerize before or after binding to the membrane? In vitro MinD dimerizes more readily if the MTS is removed. Thus, in vivo the membrane could help sequester the MTS away from the dimer interface, therefore promoting dimerization. Fluorescence resonance energy transfer (FRET) between labeled MinDs is promoted by the presence of vesicles, suggesting that dimerization is promoted by the phospholipid bilayer (107). Also, MinD interacts more strongly with itself in the yeast two-hybrid system than with MinD lacking the MTS (111). These results are consistent with monomers transiently associated with the membrane being locked on the membrane through surface-assisted dimerization (109).

MinD has also been shown to bind cooperatively to lipid vesicles (107, 112). In addition, MinD can undergo assembly on a phospholipid surface to convert membrane vesicles into tubes (103). The MinD is wrapped around the tubulated vesicle in a helical structure. The surface-dependent polymerization of MinD appears similar to the cooperative assembly of Soj on double-stranded DNA (99). Dimers once bound to the membrane could further associate to form the coils observed in vivo with GFP-MinD or to produce the tubulation of vesicles observed in vitro (75, 103). Although MinD and Soj only undergo surface-associated polymerization, other ParA members are reported to polymerize in solution (77, 113, 114).

MinD ATPase

MinD expresses a basal ATPase that is also observed with other members of the WACA family (86). MinD's ATPase is stimulated (~10-fold) by MinE in the presence of phospholipid vesicles, indicating MinD has to be bound to a membrane surface to be stimulated by MinE (115). In the monomer, the signature lysine within the deviant Walker A motif interacts with several residues within helix 7, especially D152 (101). This interaction appears unique to MinD because it is not observed in the closely related Soj, which lacks the corresponding aspartic acid residue. This aspartic acid residue is required for binding MinE in the yeast two-hybrid system and for MinE stimulation of the ATPase activity (116–118). One possible model for ATPase activation is that MinE competes with D152 for binding to the lysine, resulting in its release and ATPase activation (117). However, the D152A mutation, which would disrupt this interaction, does not lead to constitutive ATPase activity as might be expected from such a model and suggests that MinE has an additional role in the stimulation (118). The D152A mutant is activated by ADP to bind the membrane and its partners.

FRET: fluorescence resonance energy transfer

MinE

MinE is a small protein of 88 amino acids that forms a dimer (119). It has at least two activities associated with two separable functional domains (62, 120). An anti-MinCD activity is present in the N-terminal domain (residues 1–31) and can suppress the division inhibitory activity of MinCD; however, it does this without topological specificity. This anti-MinCD activity correlates with the ability of MinE to bind MinD and stimulate its ATPase activity. This is consistent with the ATP form of MinD being the active form of the division inhibitor. Thus, MinE converts MinD to the inactive form. The topological specificity of MinE, the ability of MinE to spatially restrict the activity of MinCD, requires the C-terminal domain (residues 32–88). The structure of the C-terminal domain has been determined by NMR and is responsible for dimerization of MinE (119). It is not clear if the topological specificity function of this domain is simply to dimerize the N-terminal domain or if it has additional activities. A recent study indicates that the N-terminal domain (not present in the structure) interacts with the C-terminal

domain, arguing that these domains are not completely independent (121).

MinC

MinC is the effector of the Min system responsible for antagonizing cell division (55, 60). In vitro experiments have shown that MinC antagonizes FtsZ assembly without affecting its GTPase activity (81). Biochemical and genetic studies indicated MinC is a dimer with each monomer consisting of two functional domains (82). The N-terminal domain is responsible for inhibiting cell division in vivo and antagonizing FtsZ assembly in vitro. The C-terminal domain is responsible for dimerization and interaction with MinD. A highly conserved sequence in the C-terminal domain (RGSQ) is required for the interaction with MinD (122, 123). The two domains are connected by a flexible linker that varies in length among MinCs from various bacteria. Determination of the structure of MinC from *T. maritima* confirmed that it was a dimer with each monomer consisting of two independent structural domains (124). In the crystal, the MinC dimers were observed in two conformations owing to different orientations of the N-terminal domains afforded by the flexible linker.

Although MinC is the inhibitor of cell division, it is a relatively weak inhibitor in the absence of MinD. MinD activates MinC ~25- to 50-fold (60). It does this in part by recruiting MinC to the membrane. This activation can be artificially induced by attaching MinC to the membrane. Adding membrane-anchoring sequences at the N- or C-terminal ends of MinC dramatically increases its inhibitory activity (15, 109). However, this does not appear sufficient to fully activate MinC. The MinCD complex is not only targeted to the membrane, it also acquires affinity for some septal component. This was revealed by expressing a GFP-tagged version of MinC with a defective N-terminal region in the presence of MinD and absence of MinE (125). This fusion is recruited to septal complexes, although it is relatively nontoxic. Support for the importance of this targeting to the septal complex is provided by the R172A mutation, which abrogates MinC's ability to be targeted by MinD and regulate division without affecting its ability to participate in oscillation (122). The component of the septum that is targeted by MinCD is not known but is likely to be FtsZ because targeting is still observed if FtsA or ZipA is removed (15). MinC can also be activated by DicB, which is encoded by a defective prophage in some *E. coli* strains but not usually expressed. The DicB-MinC complex does not bind the membrane but is targeted directly to the septum, in part through an interaction between DicB and the septal component ZipA.

It is interesting to compare the activity of the two different MinC complexes. In contrast to the MinCD complex, the DicB-MinC complex is not topologically regulated. It is targeted directly to septal rings, destabilizing them regardless of cellular location (125). In contrast, the MinCD complex is targeted to the membrane where it acquires affinity for septal rings. In the presence of MinE, this complex is directed to the poles of the cell, and it does not come into contact with a Z ring forming at midcell. Any septal rings attempting to form away from the cell center encounter the polar MinCD and are disrupted. The positioning of the MinCD complex to the membrane at the poles by MinE is therefore critical to its function. Binding to the membrane activates the complex, in part by concentrating MinC at the membrane and in part by bestowing affinity for a septal component (110, 125). The requirement for membrane binding of MinCD to achieve full activity along with its targeting to the poles spatially restricts its activity.

Although MinC is only a passenger in the oscillation, it is the effector of the system. The main activity of MinC, revealed by in vitro studies, is its ability to prevent the sedimentation of FtsZ in a polymerization assay (81). Also, the network of FtsZ polymers, observed by negative stain electron microscopy,

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is reduced by MinC. A direct interaction between MinC and unpolymerized FtsZ is supported by a biosensor assay, which yields a K_D of ~1 μM. Furthermore, a mutation affecting the N-terminal domain of MinC reduces this affinity. Whether this affinity increases with polymerized FtsZ is not known.

The activity of MinC in the polymerization assay is quantitatively comparable to SulA, which blocks FtsZ assembly by sequestration (81, 126, 127). SulA binds to one end of FtsZ, preventing it from participating in assembly and therefore inhibiting FtsZ's GTPase (128). In contrast, MinC has little effect on FtsZ's GTPase, indicating it does not block assembly. Although the exact mechanism of MinC's effect on FtsZ assembly is unknown, it is likely promoting disassembly and possibly preventing lateral interactions between FtsZ protofilaments. Surprisingly, the N-terminal domain of MinC (which is a monomer and does not interact with MinD) displays the same activity as the full-length version in vitro (82). The ability of MinC to prevent net assembly of FtsZ is similar to the activity of another negative regulator of Z-ring assembly, MipZ, found in *C. crescentus* (see below) (96).

Interaction of MinD with MinC and MinE

MinD binds to both MinE and MinC and recruits them to the membrane. The evidence suggests that the binding sites for these two partners overlap. In the presence of a nonhydrolyzable analogue of ATP (ATPγS), MinD will recruit either MinC or MinE to phospholipid vesicles (110, 112). If both are present, MinE is the preferred binding partner. This result indicates that MinE displaces MinC from the MinD-vesicle complex in a step preceding ATP hydrolysis. Thus, despite having overlapping binding sites on MinD, MinC does not interfere with MinE binding and stimulation of the ATPase.

The interaction between the Min proteins has also been explored using the yeast two-hybrid system. This analysis supports the view of overlapping sites. Some mutations in *minD* disrupt binding to both MinC and MinE without affecting MinD self-interaction (116–118). Other mutations primarily disrupt MinC or MinE interaction. In addition, removing the MTS from MinD does not abrogate the interaction between MinD and its partners (104, 111). This argues that MinDΔMTS binds to MinC and MinE, even though it does not bind the membrane.

MinD ATPase and Oscillation

MinD is a weak ATPase that is stimulated ~10-fold by MinE in the presence of phospholipids vesicles (115). This stimulation is complex because it displays cooperative behavior for both MinD and MinE (115, 129). The cooperativity associated with MinD (observed around 2 μM) probably reflects the cooperative membrane binding, which, in part, is probably due to a requirement for the dimerization of MinD. The basis for the cooperativity associated with MinE is uncertain. The cooperativity is observed around 1 μM MinE and may be due to the dimerization of MinE. However, MinE forms a ring at the edge of a polar MinD zone, and this may indicate that MinE dimers further associate on a MinD polymer.

The anti-MinCD activity of MinE, found in the 31 N-terminal amino acids, is the same region that is required for binding MinD (110, 116). However, an N-terminal fragment is less efficient than full-length MinE in suppressing MinCD activity, indicating it has to be present as a dimer for full activity (120). Also, N-terminally truncated MinEs are able to inhibit full-length MinEs, and this activity correlates with the ability to form heterodimers (130). Thus, a heterodimer with one of the monomers containing an N-terminal-truncated MinE has little activity compared to a wild-type dimer.

The stimulation of MinD ATPase displays remarkable similarities to the stimulation of the Soj ATPase. Soj undergoes

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ATP-dependent dimerization and binds DNA forming a nucleofilament (99). The Soj ATPase is stimulated about threefold by SpoOJ and almost 10-fold by SpoOJ in combination with DNA. Soj stimulation can be also achieved by an N-terminal fragment of SpoOJ that displays similarities to the N-terminal fragment of MinE. The SpoOJ N-terminal peptide is less than 10% as efficient as an intact SpoOJ, indicating dimerization promotes its activity.

The ability of MinE to stimulate MinD ATPase correlates with its ability to stimulate the oscillation in the Min system (115). In the absence of MinE, MinD is present throughout the membrane. Known *minE* mutations that prevent the ATPase stimulation map to the N-terminal domain and prevent binding to MinD (110, 115). Importantly, several *minE* mutations that map to the N-terminal domain show reduced stimulation of the MinD ATPase and display a reduced oscillation frequency (115). This correlation indicates that the ATPase stimulation by MinE determines the periodicity of the oscillation. In addition, a MinE mutant with two amino acids altered in the C-terminal domain induces a slow and disordered oscillation (68). The basis for this behavior is unknown. Recently, *minD* mutations that alter a charged patch on the surface of MinD have been isolated (131). The mutants still interact with MinE but are unable to stimulate the ATPase. These mutants, as expected, fail to oscillate and are distributed around the membrane. The mutants form coils in the presence of MinE, but coils are not observed in the absence of MinE.

One puzzle about the Min system is the presence of MinE in the MinD polar zone. No function has been ascribed to this localized MinE. One possibility is that MinE helps to promote MinD coils in addition to its role in destabilizing the MinD polar zone by stimulation of the ATPase. This possibility is also suggested by the apparent absence of coils in cells producing GFP-MinD in the absence of MinE (75, 131).

MODELING THE OSCILLATION

Because of the relative simplicity of the Min oscillation, only two required proteins, it has proven an attractive candidate for modeling (132–134). The models try to recapitulate the observed dynamics of the oscillation revealed with GFP fusions and ascertain critical aspects. The dynamic patterns include the MinD polar zone, the MinE ring, the pole-to-pole oscillation with a period of ∼40–50 s and the striped pattern observed in filamentous cells. MinC is not included in the models because it does not affect the oscillation, and all results demonstrate that it would simply follow MinD. Second-generation models have incorporated additional features, such as the MinD ATPase, MinD assembly into filaments, and the stochastic nature of the process with a small number of molecules per cell (135–140). Several models with variations on the general mechanism can generate the oscillations, emphasizing that the true basis of the oscillation will require experimental confirmation.

All approaches involve a set of coupled reaction-diffusion equations that represent the concentrations of MinD and MinE on the membrane and in the cytoplasm as a function of cell length and time (141). An important feature of such models is that diffusion of a protein on the membrane is much slower than in the cytoplasm. Measured rates for several proteins indicate that integral membrane proteins diffuse about 100-fold slower than a cytoplasmic protein (142).

The main feature of most of these models is that MinD binds to the membrane and recruits MinE, which ultimately induces the detachment of both proteins (**Figure 2**). These assumptions are well documented in vivo and in vitro. Most of the models have MinD binding cooperatively to the membrane, which has also been reported (107, 112). A difficult aspect of the oscillation is for the released MinD from one pole to assemble a new polar zone at the opposite pole. If MinE and MinD diffuse at the same rate, then such relocation would

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not be possible because the concentration of MinD is highest where it comes off the membrane and would likely rebind close to its release. To overcome this, several models simply have MinE coming off the membrane at a much slower rate than MinD (132–134). This recalcitrant MinE shields the old pole from MinD rebinding and therefore forces MinD to the far pole. Such behavior of MinE-GFP has not been observed.

Another approach invokes a somewhat slow nucleotide exchange by MinD to help achieve the oscillation (135). It and subsequent variations are the only models that involve, and in fact require, three dimensions. In this model, MinD and MinE come off the membrane after MinE stimulates MinD to undergo ATP hydrolysis. MinE can immediately rebind to nearby MinD, which is still attached to the membrane at the occupied pole, whereas the released MinD, now in the ADP form, must undergo nucleotide exchange (**Figure 2**). Because MinD-ATP binds preferably to membranes already containing MinD, the MinD bound to the old pole functions as a sink to lower the cytoplasmic concentration of MinD-ATP at this pole. In contrast, MinD-ATP that diffuses toward the opposite pole binds less efficiently to the membrane because it is free of membrane-bound MinD. However, as the cytoplasmic concentration of MinD-ATP at this end rises, it will eventually rebind leading to formation of a new polar zone. This model reproduces the pole-to-pole oscillation in short cells and the striped pattern in long cells. Also, the periodicity in this model is affected by the MinD/MinE ratio as shown in vivo. Because this model involves a three-dimensional cell, it was also readily adapted to Min oscillation in round cells (143).

After GFP-MinD was observed as coils that presumably represent polymers of MinD, an effort was made to incorporate polymerization of MinD into a model (136, 138). Such models were able to reproduce the oscillations; however, a number of additions had to be made for the model to achieve oscillation. In one, the filaments emanating from the pole have to grow at the same rate. This was achieved by favoring the growth of short MinD filaments. Second, it was necessary to nucleate the MinD polymers at the cell pole (136). The introduction of a nucleator removes an advantage of the other models in which the Min proteins and the membrane are sufficient to produce oscillations. This could still be the case if MinE helped to nucleate polymers as well as induce their disassembly. Otherwise, an additional component is necessary. This seems unlikely given the ability of the Min system to produce oscillation in filamentous and round cells.

MIN AND NOC WORK TOGETHER FOR SPATIAL REGULATION

Although Min has a dramatic effect on spatial control of Z-ring assembly, other factors are important. In anucleate cells, generated by interference with DNA segregation, the Z ring is positioned near midcell, but the positioning is not quite as accurate as in nucleated cells (144). Assuming this positioning reflects the activity of Min, it suggests that other factors make positioning more precise in nucleated cells. Also, in a *min* mutant, Z rings are not positioned randomly but occur at the poles and between nucleoids (7). This result suggests that nucleoid occlusion, a concept arising from the absence of septation occurring over nucleoids (145), operates at the level of Z-ring formation (7).

Proteins that are at least in part responsible for nucleoid occlusion, SlmA in *E. coli* and Noc in *B. subtilis*, are unrelated DNA-binding proteins (146, 147). Double mutants lacking Min and Noc/SlmA fail to divide, and FtsZ is distributed somewhat sporadically in arcs and rings along the filament but still preferentially between nucleoids. As described above for the combination of the Δ*min* and *ftsZ84* mutations, the distribution of FtsZ among too many structures prevents any one of them from maturing into

a functional Z ring, and cells undergo filamentous death. Consistent with this, increasing FtsZ restores viability. However, there is still preferential distribution of FtsZ between nucleoids in these double mutants, suggesting that other factors contribute to nucleoid occlusion. Noc/SlmA mutants display little phenotype during balanced growth, perhaps, stressing the importance of Min and these other factors. Also, these double mutants survive on media that support a slower growth rate. Importantly, blocking initiation of replication in a Noc/SlmA mutant leads to formation of Z rings over nucleoids and guillotining, stressing their importance under unbalanced growth.

Although the mechanism of SlmA/Noc is still not clear, several observations have been made that suggest a tentative model (**Figure 3**). Overproduction of SlmA recruits FtsZ to the nucleoid and prevents Z-ring formation, suggesting that SlmA is a negative regulator of Z-ring assembly (147). Interestingly, SlmA/Noc appears asymmetrically distributed over the nucleoid, with less near the middle of a segregating nucleoid. Also, SlmA interacts directly with FtsZ as shown by its ability to bundle FtsZ polymers. One possibility is that SlmA/Noc bound to the nucleoid competes locally with FtsA and ZipA, which attach FtsZ to the membrane. As the nucleoid segregates, a SlmA/Noc-free region at midcell may allow a Z ring to form there (147).

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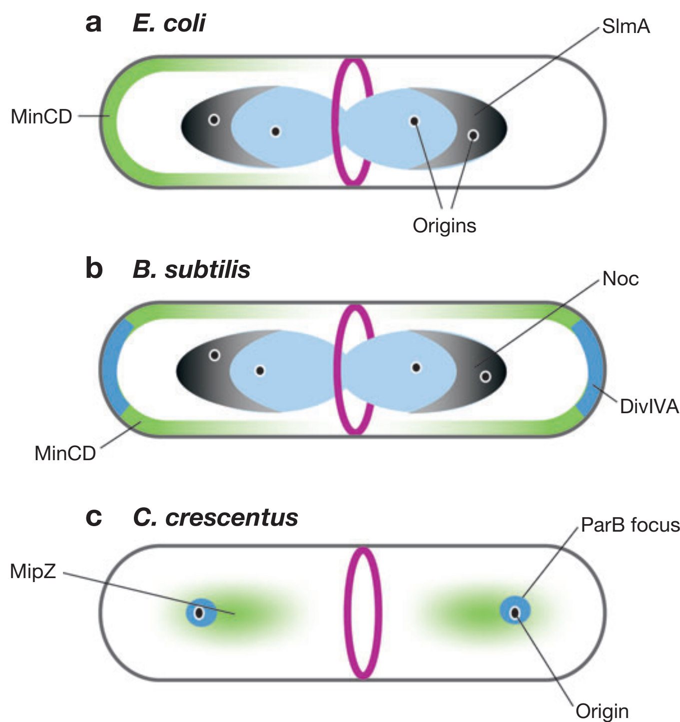

Figure 3

The Z ring is positioned by a gradient of negative regulators of Z-ring assembly. (*a*) In *E. coli*, a Z ring is positioned by a gradient of two negative regulators. One is MinC, which is established on the membrane and oscillates between the poles under the regulation of MinD and MinE (69, 70). The second is due to SlmA, which is asymmetrically distributed over the nucleoid (146, 147). (*b*) In *B. subtilis*, the Z ring is also regulated by two gradients. A gradient of MinC extends on the membrane from the poles under the regulation of MinD and DivIVA (148, 149). There is also asymmetric distribution of Noc on the nucleoids. (*c*) In *C. crescentus*, a gradient of MipZ extends from the ParB foci, located near the origins. MinC and MipZ promote disassembly of FtsZ polymers (96). The mechanism by which SlmA and Noc are asymmetrically distributed is unknown as is the mechanism by which they affect Z-ring assembly.

DIVISION REGULATION WITHOUT MIN OSCILLATION, *B. SUBTILIS*

The rapid oscillation of the Min proteins raises the issue of why there is oscillation when simple tethering of the Min proteins to the poles could achieve the same result. In fact, in *B. subtilis*, another model organism for the study of cell division, the Min proteins do not oscillate, instead MinCD spread out from the poles of the cell, resulting in a minimum at midcell (148, 149) (**Figure 3*b***). Although the pattern appears static at the cellular level, it is likely that the organization is quite dynamic at the molecular level, fueled by the MinD ATPase. This organism lacks MinE, and the polar location of MinCD requires DivIVA, which shows no sequence similarity to MinE (148). Although no direct interaction between MinD and DivIVA has been reported, DivIVA is recruited to the division site during a late stage of septation and in turn is responsible for recruiting MinD and MinC. After division, these proteins are retained at the pole,

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although DivIVA displays a more compact polar localization than MinCD.

DivIVA may be recruited to the pole by direct interaction with the division machinery; however, it is positioned at the poles of germinating spores, which lack division proteins–including FtsZ (150). Therefore, it is not clear what targets DivIVA to the cell poles. It may be that DivIVA senses the curvature of the cell membrane generated by septation. The targeting of DivIVA is polar even when expressed in *E. coli* and *Schizosaccharomyces pombe*, which do not have homologues (151). There are other differences between the *E. coli* and *B. subtilis* systems as well.

In *B. subtilis*, MinD mutants that should be deficient in ATP binding are localized to the membrane but are unable to recruit MinC (152). This is consistent with studies indicating that the MTS of *B. subtilis* MinD has a higher affinity for the membrane than that of *E. coli* and therefore does not have to dimerize to bind to the membrane (109). In such mutants, the asymmetric distribution of MinD is lost as it is distributed evenly throughout the cell membrane, but division is not inhibited because the mutants are unable to bind MinC. Thus, the binding of ATP is responsible for the polar localization of MinD and the recruitment of MinC. How this gradient of MinD, emanating from the poles, is established is not known, but it most likely involves the ATPase activity of MinD.

The position of the Z ring in *B. subtilis* is regulated by both Min and Noc (146). In the absence of Noc, cells have a normal morphology, and the Z ring is positioned at midcell. In the absence of Min, minicells are produced, and Z rings are found at the poles and between nucleoids. However, the ratio and frequency is affected by the growth rate (153). At slow growth rates, cells usually have 0 or 1 Z ring, and it is usually between nucleoids (9 medial:1 polar), whereas at fast growth rates, there are more Z rings per cell, and polar Z rings are observed at almost the same frequency as intranucleoid rings. Thus, at slow growth rates, the Z rings are preferentially positioned at midcell even in the absence of Min. This effect of growth rate favoring midcell may explain why Min and Noc/SlmA double mutants survive at slow growth rates, whereas at fast growth rates, the combination is lethal, and FtsZ is scattered between many incomplete structures (146, 147).

C. CRESCENTUS EMPLOYS A GRADIENT OF PROTEIN RELATED TO MIND

Unlike *E. coli* and *B. subtilis*, which divide to produce two nearly identical cells, *C. crescentus* undergoes division near midcell to produce two different-sized progeny cells with differing fates (154). One is a stalked cell that can immediately reenter the cell cycle, and the other is a smaller swarmer cell that does not immediately enter the cell cycle but eventually develops into a stalk cell, which occurs after some delay. *C. crescentus* lacks Min and probably the Noc system present in other bacteria. However, it has two ParA family members that are involved in spatial regulation of division. One is designated ParA and is closely related to Soj, and the other is MipZ, a more distantly related member only found among members of the α-proteobacteria (92, 96). MipZ is directly involved in spatial regulation of division in *C. crescentus* (96).

In *C. crescentus*, FtsZ expression is under cell cycle regulation; however, this regulation does not contribute to the spatial regulation of Z-ring assembly because constitutive expression does not affect the timing or positioning of the Z ring (155). However, blocking initiation of DNA replication prevents positioning of the Z ring at midcell. A Z ring still assembles, but it is positioned close to one of the poles (156). Furthermore, additional results reveal a role for the essential *parA* and *parB* genes in regulating Z-ring assembly. Depletion of ParB or overproduction of ParA prevents Z-ring assembly (93). ParB is essential, binds to ParC sites located near the origin, and appears as a focus using ParB-GFP. This focus is located at the stalked pole along

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with the origin of replication and splits in two as DNA replication is initiated. One of the foci moves to the opposite pole following initiation of replication in an MreB-dependent manner (96, 157). This segregation of the ParB foci precedes Z-ring assembly at midcell (93).

How does ParB affect Z-ring positioning? A tour de force from the Shapiro lab (96) suggests that ParB is involved in establishing a gradient of MipZ, an inhibitor of Z-ring formation. Bioinformatics and cell cycle expression studies led them to investigate a gene ultimately designated *mipZ*. This gene is essential, and increasing the level of MipZ blocks Z-ring assembly, implicating MipZ as an inhibitor of division. During the cell cycle, MipZ localizes as a diffuse focus that overlaps the ParB focus. Depletion of ParB results in redistribution of MipZ throughout the cell and a block to division. This delocalization of MipZ indicates that MipZ activity is spatially regulated by ParB. Furthermore, MipZ interacts directly with ParB as revealed by biosensor assays and the ability of MipZ to supershift a complex of ParB bound to ParC.

Further study of MipZ revealed that it directly affects FtsZ polymerization in vitro. In the presence of MipZ and ATP, the quantity of FtsZ polymers is reduced, and those present are short and curved, suggesting that MipZ induces the breakdown of FtsZ polymers. Curved polymers are associated with the GDP form of FtsZ, whereas the GTP form of FtsZ is associated with straight polymers. Importantly, a catalytically inactive mutant of MipZ is able to do this as well, indicating ATP hydrolysis is not required. In vivo this catalytically inactive mutant does not localize to the ParB foci but is distributed throughout the cell and constitutively inhibits division. These results indicate that the inhibitory action of MipZ is restricted by the ParB foci and that the ATP-form of MipZ is the inhibitor.

Simultaneous monitoring of FtsZ and MipZ with fluorescent fusions supports their roles (**Figure 3*c***). Following division, some FtsZ is retained at the nascent pole, which is opposite the stalked pole where the origin is located. Following origin duplication, one of the origins segregates to the opposite pole, inducing release of FtsZ. This FtsZ, along with newly synthesized FtsZ, forms a ring midway between the two origins owing to the MipZ gradient formed between the two ParB foci. How this gradient is formed is not clear, but it requires the ATPase activity of MipZ.

WHY DO THE *E. COLI* AND *B. SUBTILIS* SYSTEMS APPEAR MORE COMPLEX THAN *C. CRESCENTUS?*

Spatial regulation of cell division involves control over the positioning the Z ring. In the three bacteria that have been studied in some detail, important components of this regulation are gradients of inhibitors of FtsZ assembly. A comparison of the activity of these inhibitors reveals a common mechanism of action, at least to the extent we understand their mechanism. Both MinC and MipZ prevent accumulation of FtsZ polymers without inhibiting the GTPase activity. This result indicates that they induce the disassembly of FtsZ polymers. In the case of MipZ, the GTPase is stimulated slightly, and MipZ addition is associated with the presence of short curved polymers, instead of the longer straight polymers typically seen.

The spatial regulation of Z-ring assembly observed in *C. crescentus* provides a direct link between chromosome segregation and Z-ring positioning, ensuring that the Z ring forms between the segregated chromosomes. The segregated origins, along with the associated ParB foci, established a gradient of MipZ emanating near the poles of the cell. A Z ring forms equidistant between the origins where the MipZ concentration is lowest. Thus, ParB, bound near the origins, sets up the MipZ gradient, ensuring that the Z ring is equidistant. Many questions remain, however, especially the biochemical details of how the gradient is established and the involvement of the ATPase activity of MipZ. Also, some

adjustment to this model may be required, since the Z ring is positioned asymmetrically to produce the different-sized progeny cells.

Why don't *E. coli* and *B. subtilis* have an inhibitor gradient linked directly to the origin of replication? The answer may lie in the way the initiation of DNA replication is controlled. In *C. cresecentus*, one round of replication is completed, and the cells divide before another round is initiated. Thus, cells have either one or two origins. In contrast, at moderate growth rates in *E. coli* and *B. subtilis*, initiation of replication can occur before the first round is complete, and a cell can have more than two origins. At even faster growth rates, the number of origins per cell increases, and a single cell can have 8–16 origins. This multifork replication is an evolutionary solution that allows these bacteria to achieve a 20-min generation time when it takes 40 min to replicate the chromosome (158). Thus, it might be difficult to use the origin as a marker; there are just too many.

Instead, the Min system has evolved to find the center of the long axis of a cylinder while ignoring the DNA as a spatial marker. This is an effective system to prevent Z-ring formation at the poles but is not a system to coordinate Z-ring formation with segregation of the nucleoid. To do this, *E. coli* and *B. subtilis* have a separate system that ensures that the chromosome is mostly segregated before the Z ring is assembled. This system is still not well understood but involves Noc/SlmA binding to the DNA. As the nucleoid segregates, SlmA/Noc appears asymmetrically distributed with more at the edge of the nucleoid near the pole. This nucleoid-induced distribution of SlmA/Noc away from midcell may allow the Z ring to form there. Thus, the two systems, acting together, form a negative regulatory system that prevents Z rings from forming at the poles and over the nucleoid.

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SUMMARY POINTS

1. The Z ring is a cytoskeletal element that recruits additional division proteins to form a septal ring that carries out cytokinesis in bacteria.
2. The position of the Z ring is determined by gradients of negative regulators of Z-ring assembly, which have a minimum at midcell. A variety of negative regulators can be used by a bacterium, and different bacteria use the same or different regulators.
3. Two of these negative regulators, MinC and MipZ, promote the disassembly of FtsZ polymers.
4. In *E. coli*, a gradient of MinC is formed on the membrane by an oscillatory mechanism that is determined by MinD and MinE. In *B. subtilis*, a static gradient of MinC on the membrane is formed by DivIVA and MinD.
5. The basis for the dynamic behavior of the Min proteins in *E. coli* is MinE stimulation of the MinD ATPase. MinD binds to the membrane in an ATP-dependent manner and recruits MinC and MinE. MinE displaces MinC and stimulates the MinD ATPase, releasing the proteins from the membrane.
6. Computer simulations can reproduce the oscillatory behavior of the Min proteins by incorporating the known behavior of the proteins.
7. In *C. crescentus*, ParB bound near the origin of replication is involved in establishing a gradient of MipZ.

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FUTURE ISSUES

1. The structure of the Z ring needs to be elucidated. How are polymers of FtsZ arranged in the Z ring? What role do accessory proteins play in maintaining the ring?
2. The mechanism of forming the gradients of MinCD in *B. subtilis* and of MipZ in *C. crescentus* remains to be determined. What roles do the ATPase activities of MipZ and MinD play? How are the ATPases regulated?
3. How do the regulators of nucleoid occlusion, SlmA and Noc, affect Z-ring assembly? And how is their asymmetric distribution over the nucleoid established? What other factors are involved in nucleoid occlusion, especially at slow growth rates, which allow Min/Noc (SlmA) double mutants to survive?
4. Evidence is accumulating that members of the ParA family polymerize. MinD and Soj undergo surface-dependent assembly (Soj on DNA and MinD on vesicles), whereas other members appear to polymerize in solution. These assemblies need to be better characterized.

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