Bacterial Cell Division:
The Cycle of the Ring

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The bacterial cell cycle can be arbitrarily divided into two segments: a DNA cycle that includes DNA replication and chromosome segregation, and a division cycle that leads to cytokinesis and cell separation. During the division cycle, the cell must identify the midcell site at which division later occurs, differentiate this site in preparation for cytokinesis, and finally form the division septum by the coordinate ingrowth of the cytoplasmic membrane, the rigid murein (peptidoglycan) layer, and, in Gram-negative bacteria such as Escherichia coli, the outer membrane of the cell envelope (Figure 1). Recent advances have led to an increased understanding of important elements of this complex series of events.

Many, although probably not all, of the proteins involved in the division cycle of E. coli are now known. One of these proteins, FtsZ, is now recognized to play a key role in the assembly of the division apparatus and in the process of cytokinesis. Its wide distribution and high degree of sequence conservation suggest that it probably plays a similar role in all bacterial and archaeal species. In this minireview, we discuss the likely sequence of events that occurs during differentiation of the division apparatus of E. coli, beginning with the localization of FtsZ at the potential division site and ending with the generation of two new daughter cells (Figure 1).

Stage I. FtsZ-Receptor Binding

Studies by Lutkenhaus and his collaborators (Lutkenhaus and Mukherjee, 1996) have established that FtsZ becomes localized at the future division site at an early stage in the division cycle and remains associated with the invaginating septum during cytokinesis. Because FtsZ acts at an earlier stage of the division process than the other known components of the division machinery, with the possible exception of ZipA (see below), the interaction of FtsZ with the cytoplasmic membrane at the future division site is an obligatory early step in the sequence of events that culminates in septation and cell separation.

The site-specific membrane recognition event implies the presence of a specific FtsZ receptor at midcell (Figure 1). Although the receptor has not yet been identified, we may be closing in on the prey. In a recent issue of Cell, Hale and de Boer (1997) described a newly identified integral membrane protein, ZipA, that emerged from a direct search for genes coding for proteins that interact with FtsZ. ZipA has several properties expected of the FtsZ receptor and the evidence indicates that the ZipA–FtsZ interaction occurs at the midcell site at a very early stage of the division cycle. First, ZipA is an integral membrane protein that binds FtsZ in vitro. Second, ZipA is required for cell division as shown by the formation of nonseptate filaments in the absence of ZipA. The zipA null filaments morphologically resemble ftsZ null filaments, rather than the filaments associated with loss of other division genes that act at later stages of the division cycle. Third, in a growing population, a ZipA–green fluorescent protein (GFP) fusion protein is localized at midcell in essentially all cells. Finally, the distribution pattern of ZipA–GFP at the division site is similar to that of FtsZ, with ZipA being located in a ring that extends around the circumference of the cell. These observations indicate that ZipA is the FtsZ receptor or interacts with FtsZ concurrently or soon after the initial FtsZ–membrane interaction. A choice between these possibilities awaits the results of experiments to establish whether or not FtsZ can localize to the midcell site in the absence of ZipA.

Stage II. Formation of the FtsZ Ring

In a reaction that probably occurs shortly after its initial interaction with the membrane, the membrane-associated FtsZ forms an annular structure, the FtsZ ring, that is associated with the inner surface of the cytoplasmic membrane and extends around the circumference of the cell (Lutkenhaus and Mukherjee, 1996). It is believed that the FtsZ ring is produced by polymerization of FtsZ protomers. This is consistent with the observation that FtsZ can be induced to polymerize in vitro, in a reaction that is facilitated by GTP, leading to the formation of filamentous polymers whose protofilament structure is similar to that of microtubules (Ericsson et al., 1996). There are 10,000–20,000 molecules of FtsZ per cell, enough to produce several continuous polymeric structures around the waist of the cell. The ring may consist of one or more polymers that extend completely around the cell, or it may be a series of shorter polymers organized to form a continuous annulus. FtsZ isolated from the soluble fraction of the cell exists as an oligomer whose gel filtration behavior suggests a size of 10–15 monomers per oligomer. This is presumed to be the

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Figure 1. Proposed Sequence of Events during the E. coli Division Cycle

See text for details. OM, outer membrane; Mur, murein (peptidoglycan) layer of the cell envelope; CM, cytoplasmic membrane; R, hypothetical FtsZ receptor.
species that binds to the receptor and acts to nucleate FtsZ polymerization.

The initial binding of FtsZ could occur at multiple sites within the preseptal domain at midcell, with multifocal polymerization occurring from each of these sites to account for formation of the final ring. However, recent immunofluorescence experiments on the spherical cells of rodA mutants have shown that in some cells FtsZ is present as an arc, or partial annulus, that does not extend completely around the cell circumference (Addinall and Lutkenhaus, 1996b). This suggested that FtsZ may first interact or initiate polymerization on one side of the cylinder. The ring would then be extended around the cylinder to form the complete annular structure that is seen in most cells. If the FtsZ pattern of the rodA mutant cells reflects the pattern of ring formation in wild-type cells, the initial FtsZ–membrane interaction might only require a small number of receptors that are clustered at one site.

**Stage III. Assembly of the Cytokinesis Machinery**

In addition to FtsZ and ZipA, at least seven additional protein components are required for cytokinesis. These include one peripheral membrane protein (FtsA) and six integral cytoplasmic membrane proteins (FtsI, FtsQ, FtsL, FtsW, FtsN, and FtsK). In the case of FtsA, there is direct evidence that the protein is localized at the division site. For the others, the evidence is thus far inferential.

FtsA differs from most of the other membrane-associated division proteins in its abundance (several hundred copies per cell for FtsA versus fewer than 50 copies per cell for most of the other proteins) and its apparent peripheral membrane association. FtsA recovered in the soluble fraction of broken cells is apparently phosphorylated whereas the membrane-associated FtsA is not (Sanchez et al., 1994). It is not yet established whether FtsA phosphorylation plays a role in assembly or function of FtsA since an unphosphorylated form of the protein is still capable of complementing the cell division defect in an ftsAts mutant (Sanchez et al., 1994).

The distribution of FtsA in a circumferential ring at the division site, as shown by immunofluorescence studies and by examination of an FtsA-GFP chimera, is similar to that of FtsZ (Addinall and Lutkenhaus, 1996a; Ma et al., 1996). This pattern of FtsA localization requires a functional FtsZ protein since the FtsA ring was not seen when formation of the FtsZ ring was prevented by mutation of ftsZ or by expression of the SulA (SfiA) division inhibitor. As expected, FtsZ ring formation was not prevented in ftsA filaments. Support for the view that the localization of FtsA to the division site is dependent on FtsZ has also come from studies of ftsZ26 mutant cells, in which a mutation in the FtsZ coding sequence results in a spiral pattern of septum formation and a concomitant spiral pattern of the FtsZ ring (Addinall and Lutkenhaus, 1996b). FtsA distribution in these cells showed the same spiral distribution. Thus, FtsA localization depends on direct interaction of FtsA with FtsZ or with another division component whose localization is FtsZ-dependent. Whether FtsA moves to the membrane together with FtsZ or after FtsZ binds at the division site is not known.

Interaction between FtsA and FtsZ is also suggested by the observation that division is blocked by overexpression of either FtsA or FtsZ, and in each case the division block can be reversed by the concomitant overexpression of the other protein (Lutkenhaus and Mukherjee, 1996). Although not direct proof of protein–protein interaction, the requirement for a certain FtsZ/FtsA ratio for proper assembly or function of the division apparatus is consistent with the direct interaction of the two proteins.

The other known division proteins (Ftsl, FtsQ, FtsL, FtsW, FtsK, and FtsN; Begg et al., 1995; Dai et al., 1996; Lutkenhaus and Mukherjee, 1996) are transmembrane proteins. Formation of the FtsZ ring was not prevented in ftsAts, ftsItlts, or ftsQts mutants (Addinall et al., 1996), indicating that formation of the FtsZ ring precedes or occurs independently of assembly of these components of the division apparatus. It is not known whether assembly of Ftsl, FtsQ, FtsL, FtsW, FtsK, and FtsN into the division machinery depends on the prior assembly of the FtsZ ring, although the spiral pattern of septal ingrowth in the ftsZ26 strain (see above) suggests that the organization of the entire division apparatus may be determined by the pattern of FtsZ organization within the ring.

Ftsl, FtsQ, FtsL, and FtsN are bitopic proteins that cross the lipid barrier once. Each has a large periplasmic domain, suggesting that these proteins participate in division-related functions that occur outside the cytoplasmic membrane.

Ftsl is a murein biosynthetic enzyme that is required for formation of the murein layer of the division septum but not for murein synthesis during cell elongation. Since the murein layer lies outside of the cytoplasmic membrane, the role of Ftsl is almost certainly to catalyze the synthesis of septal murein within the periplasm. Although its periplasmic domain is essential for Ftsl function, the transmembrane domain and small cytoplasmic domain are also required since they can not be replaced by the corresponding domains of the unrelated MalG inner membrane protein without loss of function (Dai et al., 1996). Whether the transmembrane and cytoplasmic domains of Ftsl are needed only for its proper assembly into the division apparatus, or whether they play a more direct role in its division-related function is unknown.

Domain-swapping studies of FtsQ have confirmed the essential role of its large periplasmic domain as shown by the observation that chimeric FtsQ proteins in which the membrane-spanning and cytoplasmic domains of FtsQ were replaced by the corresponding regions of MalG were still capable of restoring division function to a temperature-sensitive ftsQ strain (Dai et al., 1996). The function of FtsN also appears to reside in its periplasmic domain since a soluble periplasmic form of FtsN is sufficient to restore division activity to an ftsN null mutant (Dai et al., 1996). Surprisingly, overexpression of FtsN suppresses the division defect of certain ftsA and ftsI mutants. This suggests that FtsN may play a role in assembly or stability of the septation machinery, or possibly in the indirect regulation of expression some division genes.

**Stage IV. Cytokinesis**

The process of septal invagination is complex, involving the circumferential ingrowth of the three layers of the cell envelope—the cytoplasmic membrane, the murein
layer, and the outer membrane. Two models could explain this process. In the PUSH model, a cell cycle signal is transmitted via the division apparatus to the septal murein-synthesizing machinery at the midpoint of the cell. Ingrowth of the rigid murein layer at right angles to the long axis of the cell then “pushes” the cytoplasmic membrane inward. In the PULL model, cytokinesis is initiated by constriction of the FtsZ ring, which “pulls” on the cytoplasmic membrane, thereby transmitting a signal to the septal murein-synthesizing machinery to begin synthesis of septal murein. In attempting to distinguish between these models, it is significant that FtsZ homologs are present in microorganisms that do not contain murein, such as Archaea and Mycoplasma (Margolin et al., 1996; Wang and Lutkenhaus, 1996a, 1996b). If the ftsZ gene products in these organisms are part of their division apparatus, as one would suspect, this suggests that constriction of the FtsZ ring during cell division can occur independently of murein ingrowth, arguing against a simple PUSH model of bacterial cytokinesis. It therefore is likely that constriction of the FtsZ ring is a key element in septum formation, with septal murein ingrowth being a secondary or perhaps parallel process that responds to the same signal that triggers constriction of the ring.

Constriction of the FtsZ Ring. Immunoelectronmicroscopy has shown that the FtsZ ring remains associated with the leading edge of the septum throughout septal ingrowth but is absent from the newly formed poles of the progeny cells (Lutkenhaus and Mukherjee, 1996). Therefore, mechanisms are required both to cause ring constriction and to eliminate FtsZ molecules from the septal site by the time that septal closure and cell separation occur.

We consider two models to explain these observations. In one model, the progressive shortening of the FtsZ ring would occur by the progressive extrusion of individual FtsZ subunits from the ring into the cytoplasm with the concomitant reformation of the end-to-end association of the remaining subunits of the ring. An attractive feature of this model is its coupling of the two observed phenomena, constriction of the ring and loss of FtsZ molecules from the division site. In an alternative model, based on the similarity of FtsZ protofilaments formed in vitro to microtubule protofilaments (Erickson et al., 1996), the FtsZ ring would behave like an array of microtubules or actin filaments, providing a track that is traversed by a motor protein that is responsible for the progressive shortening of the ring during cytokinesis. The motor could accomplish this, for example, by providing the motive force for a sliding filament reaction in which segments of the ring slide past each other to progressively shorten the circumferential structure in a manner similar to muscle contraction. Another mechanism would then be required to account for loss of FtsZ from the division site by the time of termination of septation. If this proves to be the mechanism of ring contraction, FtsA is a candidate to be a component of the motor since FtsA contains a predicted nucleotide-binding domain and has been shown to react with an ATP-agarose column (Sanchez et al., 1994), suggesting that FtsA function might involve an energy-dependent step.

Ingrowth of Septal Murein. In a plausible scenario that couples septal murein synthesis with other events of cytokinesis, a signal provided by the initiation of FtsZ ring contraction is transduced to FtsI and possibly other proteins involved in the synthesis of septal murein, using one or more of the other division proteins as signal transducers. FtsA may be involved in the coupling mechanism since it has been shown that the penicillin-binding activity of the presumed septal murein synthetase, FtsI, is significantly diminished in an ftstA mutant (Tormo et al., 1986). Invagination of Outer Membrane. It is known that the ingrowth of outer membrane can be dissociated from ingrowth of the septum by mutations that affect the attachment of the murein layer to the outer membrane (Fung et al., 1978). These include lkyD mutations, which interfere with the covalent attachment of the murein layer to the Lpp lipoprotein of the outer membrane, and deletions of the lpp gene (previously called ipo). In both cases the outer membrane fails to invaginate normally into the new septum, suggesting that outer membrane is a passive passenger that is pulled inward into the nascent septum by the ingrowth of septal murein.

Some Speculations and Caveats

The FtsZ ring plays a central role in all current models of the division process. It is often assumed that the ring consists of a single FtsZ polymer or a series of FtsZ polymers that interact noncovalently to form a continuous structure, held together primarily by interactions between FtsZ molecules. Although this may be correct, it should be noted that the evidence for a continuous ring comes exclusively from fluorescence microscopy and immunoelectron microscopy, relatively low resolution techniques. Similar microscopic studies have now shown that two other proteins, ZipA and FtsA, also appear as rings at the midcell site. It is not known how these proteins are distributed within the ring. For example, ZipA and/or FtsA might provide links between extended FtsZ polymers, or the “ring” itself might be discontinuous, with segments of cytoplasmic membrane interspersed between regions containing FtsZ and the other cytoplasmically exposed proteins of the division machinery. Models that depend on the cytokinetic ring being a continuous FtsZ structure, including those suggested in this minireview, may require revision when the molecular structure of the cytokinetic ring becomes known.

Although a great deal of work remains to be done, the organization of the division apparatus is beginning to emerge from the fog, to a point where the key questions about the assembly process and the mechanism of cytokinesis can be posed in molecular terms. However, it is striking that we still know nothing about what are perhaps the most intriguing questions: how does the cell identify its midpoint and then alter this site to provide a landing pad for FtsZ, and what explains the temporal control of cytokinesis?

After the division machinery is assembled, the septum is formed over approximately a ten-minute period that begins a short time after the daughter nucleoids are segregated to opposite ends of the cell. The mechanism responsible for the tight control of the timing of this event remains a mystery. Immunoelectronmicroscopy originally suggested that FtsZ moves from cytosol to
the division site at or shortly before the initiation of septal invagination (Lutkenhaus and Mukherjee, 1996). This suggested that a cell cycle–specific event triggered the initial interaction of FtsZ with the division site, and that this interaction in turn was the proximate stimulus for the subsequent events of septasome assembly and septal morphogenesis. More recently, the more sensitive technique of fluorescence microscopy has shown that the midcell FtsZ ring is not restricted to cells that are about to initiate septal ingrowth and that FtsZ is, in fact, visible at midcell in over 90% of the cells in the population (Addinall et al., 1996). Thus, the movement of FtsZ from cytosol to membrane occurs long before the onset of cytokinesis. Because these studies were done in rapidly growing cells in which the chromosome replication event that leads to formation of the daughter nucleoids begins during the preceding division cycle, it cannot yet be decided whether the movement of FtsZ to the midcell site is temporally related to a cell cycle event such as initiation of chromosome replication or whether it occurs constitutively, requiring only the presence of the appropriate receptor site. In the latter case, a positive signal (or relief of a negative signal) would be needed to initiate cytokinesis after assembly of the division apparatus is completed and chromosomes have been replicated and segregated.

The fact that FtsZ and ZipA rings are visible at midcell in most cells in a growing population, including cells prior to completion of cell separation (Hale and de Boer, 1997) and shortly after division (Pogliano et al., 1997), suggests that the newborn cell may already contain a nascent division site at midcell that is competent to interact with FtsZ. This is consistent with previous studies indicating that the periseptal annuli that later flank the division septum are already present at the midpoint of newborn cells (Figure 2). Similar structures may also be present at the cell quarters of predivision cells, as shown by increased sensitivity to plasmolysis at these sites. This led to the suggestion that the differentiation of the future division site may begin during the preceding division cycle (Cook and Rothfield, 1994). The recent observations that newborn cells are probably competent to form the cytokinetic ring is consistent with this hypothesis. If these speculations prove to be correct, it will require that a mechanism exist to identify the cell quarters in predivision cells. One might imagine, for example, that a diffusible factor released simultaneously from the poles and from the already differentiated site at midcell (Figure 2) could play a role in identifying the cell quarters. A major challenge for the future will be to learn when and how the future division site is first identified, and is then committed to the division pathway.

Selected Reading


Figure 2. Periseptal Annuli during the Division Cycle

Each annulus is a continuous zone where the inner membrane is closely apposed to the murein–outer membrane layers of the cell envelope (Cook et al., 1986). The annuli may serve as diffusional barriers to segregate periplasmic domains at division sites and cell poles (Foley et al., 1989).