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The New Bacterial Cell Biology: Moving Parts and Subcellular Architecture

Review

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Recent advances have demonstrated that bacterial cells have an exquisitely organized and dynamic subcellular architecture. Like their eukaryotic counterparts, bacteria employ a full complement of cytoskeletal proteins, localize proteins and DNA to specific subcellular addresses at specific times, and use intercellular signaling to coordinate multicellular events. The striking conceptual and molecular similarities between prokaryotic and eukaryotic cell biology thus make bacteria powerful model systems for studying fundamental cellular questions.

Introduction

For decades, bacteria have been appreciated as powerful model systems for studying the basic principles of molecular biology. Much of our knowledge of eukaryotic replication, transcription, translation, and DNA repair comes through analogy with the well-characterized bacterial versions of these processes. At the cellular level, however, the combination of small size and apparent lack of membrane bound organelles made bacteria appear to be homogenous, static structures whose development and organization fundamentally differed from eukaryotes. Indeed, how could such simple species ever help us understand the wondrous complexity of our own cells? Thus, despite being excellent models for eukaryotic molecular biology, bacteria were historically viewed as poor models for eukaryotic cell biology.

This traditional perspective changed significantly in the past decade with dramatic advances in our understanding of bacterial cell biology. Work in multiple species has demonstrated that bacteria are actually highly ordered and dynamic cells. Much like their eukaryotic counterparts, bacterial cells are capable of polarizing, differentiating into different cell types, and signaling to each other to coordinate multicellular actions. The more recent surprises come from advances in fluorescence microscopy, demonstrating that bacterial cells exhibit a high level of intracellular organization. Bacteria dynamically localize proteins, DNA, and lipids to reproducible addresses within the cell and use this dynamic organization to tightly regulate complex cellular events in both space and time.

The molecular components regulating this subcellular organization often resemble those that mediate the organization of the eukaryotic cell, including cytoskeletal elements and kinase signaling cascades. In cases in which these proteins are conserved from prokaryotes to eukaryotes, bacteria can clearly serve as classical model systems, with their molecular details informing upon their eukaryotic counterparts. However, the cases in which these proteins' sequences are not conserved are often equally informative. There are only so many ways that cells have found to accomplish such universal tasks as division, polarization, and chromosome segregation. If systems are similar due to convergent evolution, they can point us toward nature's optimal solution to a problem, whereas, if they differ due to divergent evolution, they can identify the basic rules that have remained intact. These conceptual model systems (as opposed to the classical molecular model systems) thus highlight the fundamental principles and logic of biology. In this review, I attempt to summarize our current understanding of bacterial cell biology with a focus on its relevance as a conceptual model system for exploring structure/function relationships in simple living cells.

Bacteria Have Homologs of the Eukaryotic Cytoskeleton

Cytoskeletal elements must be included in any discussion of cell biology, as they represent the key regulators and executors of virtually every eukaryotic cellular process, be it cellular morphogenesis, division, differentiation, or macromolecular trafficking. The perceived absence of cytoskeletal networks in bacteria was once considered a defining distinction between prokaryotic and eukaryotic cells. Bacteria do not possess an obvious need for a cytoskeleton: they maintain their shapes with a rigid cell wall that serves as an exoskeleton, and their small size enables molecules to diffuse across bacterial cells at rates that should obviate the need for active transport. Moreover, the sequencing of large numbers of bacterial genomes (186 at time of writing, according to TIGR) did not unearth any predicted proteins that were clearly identifiable as cytoskeletal by primary sequence. This erroneous perspective has since been completely overturned with the identification of bacterial homologs of all three of the major classes of eukaryotic cytoskeletal proteins: FtsZ is a tubulin homolog, MreB and ParM are actin homologs, and CreS (crescentin) appears to be an intermediate filament protein (Figure 1).

FtsZ is a GTPase that is essential for cytokinesis and localizes to the septum of dividing bacteria (Figure 1) (Bi and Lutkenhaus, 1991). Despite their weak sequence homology, the similarity between FtsZ and tubulin was confirmed by their strikingly similar crystal structures (Lowe and Amos, 1998). FtsZ is found in virtually all bacteria, archea, and chloroplasts and acts as the central organizer of prokaryotic cytokinesis, though the specific mechanism by which the FtsZ ring contracts remains unknown (Lutkenhaus and Addinall, 1997). FtsZ is the earliest protein known to localize at

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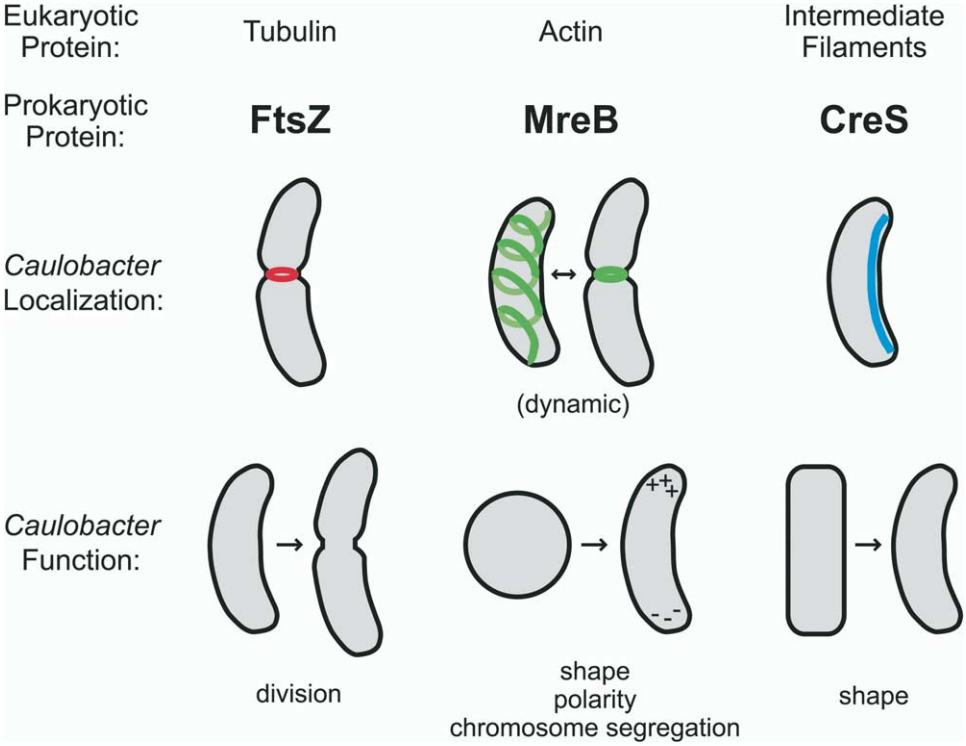

Figure 1. The Components of the *Caulobacter* Cytoskeleton

Caulobacter cells have homologs of each of the three major eukaryotic cytoskeletal systems. FtsZ is a tubulin homolog that localizes to the division plane and regulates cell division. MreB is an actin homolog that localizes to a dynamically contracting and expanding spiral and regulates cell shape, polarity, and chromosome segregation. CreS (crescentin) is an intermediate filament protein that localizes to *Caulobacter*'s inner curvature and regulates cell shape.

the division plane, acting as the most upstream member of a hierarchical localization pathway that sequentially recruits other cytokinetic factors (Errington et al., 2003). Although FtsZ protofilaments have not been shown to assemble into higher-order microtubule-like structures, FtsZ and tubulin polymerization are mechanistically related: both FtsZ and tubulin protofilaments exhibit GTP-dependent filament formation with similar dynamics and morphology (Lowe et al., 2004). Several FtsZ regulators have been identified that are similar in function but not sequence to the eukaryotic microtubule-associated proteins (MAPs) that regulate microtubule polymerization and dynamics. ZapA promotes FtsZ assembly at the division plane, while EzrA, MinC, and SulA inhibit FtsZ polymerization (Romberg and Levin, 2003). Since recombinant eukaryotic tubulin is difficult to obtain, several FtsZ mutagenesis studies served to confirm models for the mechanism of tubulin polymerization (Lowe et al., 2004). Such experiments demonstrate how the technical power of bacterial systems can be harnessed to ask questions that proved inaccessible in eukaryotes.

The identification of FtsZ as the bacterial tubulin was followed by the discovery of bacterial actin homologs. We now know of two classes of actin homologs in prokaryotes: most bacterial genomes encode one or more MreB-like actin homologs, whereas several extrachromosomal plasmids encode a different, ParM-like actin homolog (Gerdes et al., 2000). Actin, MreB, and ParM have all been shown to polymerize in vivo and in vitro and share a highly conserved tertiary structure despite their dissimilar primary sequences (van den Ent et al., 2001; van den Ent et al., 2002). The evolutionary relationships between actin, MreB, and ParM remain unclear, as they are similarly divergent from each other.

MreB proteins are found in most (but not all) nonspherical bacteria. MreB regulates cell shape by directing the localization or activity of enzymes that synthesize and reorganize the peptidoglycan making up the cell wall (Daniel and Errington, 2003; Figge et al., 2004). Also consistent with MreB playing a role in cell wall integrity, several of the *mreB* mutant phenotypes in *Bacillus subtilis* can be ameliorated by specific growth conditions (Formstone and Errington, 2004). Whereas *Escherichia coli* and *Caulobacter crescentus* each have only one MreB homolog, *B. subtilis* has three MreB homologs (MreB, Mbl, and MreBH) that have different localizations and functions (Jones et al., 2001; Soufo and Graumann, 2003). All MreB homologs examined assemble into spirals that run along the length of the cell (Figge et al., 2004; Gitai et al., 2004; Jones et al., 2001; Shih et al., 2003). *Caulobacter* MreB is also dynamic: during the cell cycle, it condenses from a spiral into a division plane-associated ring and then expands back into a spiral, hinting that MreB may do more than just regulate cell shape (Figge et al., 2004; Gitai et al., 2004)

(Figure 1). Indeed, *Caulobacter* MreB does serve other functions, acting as a global polarity determinant required for the localization of at least four different polar proteins to their correct subcellular positions (Gitai et al., 2004). MreB has also been implicated in chromosome segregation in *E. coli*, *B. subtilis*, and *Caulobacter* (Gitai et al., 2005; Kruse et al., 2003; Soufo and Graumann, 2003). The mechanisms by which MreB directs protein and chromosome localization is not known, but MreB's localization to a lengthwise "track-like" structure makes it tempting to speculate on a direct role for MreB in macromolecular trafficking. Currently, no MreB-interacting proteins have been characterized, though in many bacteria the *mreB* gene is found in an operon with two other genes, *mreC* and *mreD*, which represent putative MreB interactors (Kruse et al., 2005). MreB polymerization kinetics have not yet been investigated, but the crystal structure of *Thermotoga maritima* MreB suggests that MreB forms single linear filaments, as opposed to actin's helix of two coiled protofilaments (van den Ent et al., 2001). The future mechanistic analysis of MreB and its associated factors (motors, nucleators, capping proteins, etc.) should deepen our understanding of MreB's cellular functions.

ParM represents the other class of bacterial actin homologs and is part of a plasmid-specific mechanism to ensure faithful plasmid segregation (Gerdes et al., 2004). ParM is in an operon with a DNA binding protein, ParR, and a *cis*-acting DNA sequence, *parC*. ParR proteins bind the *parC* regions of two plasmids, and this complex promotes ParM filament formation in between the plasmids, pushing them apart (Moller-Jensen et al., 2003). No other factors are known to be required, making the ParM system the simplest known mitotic machinery and the best-understood mechanism for bacterial DNA segregation. Surprisingly, a recent study of ParM assembly demonstrated that, unlike actin filaments that preferentially polymerize at one end, ParM filaments polymerize in a symmetric, bidirectional fashion (Garner et al., 2004). ParM filaments also exhibit dynamic instability (switching between phases of steady elongation and rapid disassembly), raising the possibility that ParR promotes ParM assembly by stabilizing the ends of dynamic ParM filaments. Though ParM is an actin homolog, dynamic instability is a characteristic trait of tubulin but not actin filaments (Garner et al., 2004). Potentially unearthing a core cell biological principle, the convergent evolution of ParM and tubulin polymerization dynamics implies that dynamic instability may be an essential property of any DNA segregation machinery that must be alternately assembled and disassembled during the cell cycle.

Though the cytoskeleton mediates cytokinesis and chromosome segregation in both bacteria and eukaryotes, their functions are switched. Cytokinesis is driven by the actin-based contractile ring in eukaryotes and by the FtsZ tubulin homolog in bacteria, while DNA segregation uses the microtubule-based spindle in eukaryotes and the MreB and ParM actin homologs in prokaryotes (Gerdes et al., 2004). This apparent inversion of actin and tubulin functions could represent convergent evolution: perhaps the last universal common ancestor of bacteria and eukaryotes had both actin and tubulin but did not yet dedicate them to specific functions. Alternatively, an inversion of actin and tubulin function may have occurred in one of the two lineages. The former model suggests that the mechanisms underlying such fundamental processes as cell division and chromosome segregation independently evolved in prokaryotes and eukaryotes, whereas the latter model suggests a surprising degree of plasticity for such highly ordered and regulated multiprotein machineries. Addressing this conundrum may thus prove central to our view of the evolution of both the prokaryotic and eukaryotic kingdoms.

The most recently discovered member of the bacterial cytoskeletal family is crescentin, a *Caulobacter* coiled-coil protein whose biochemical properties and domain structure resemble those of intermediate filaments (IFs) (Ausmees et al., 2003). Like animal IFs, crescentin polymerizes in a nucleotide-independent fashion and regulates cell shape (Ausmees et al., 2003). Crescentin polymerizes on the inner curvature of comma-shaped *Caulobacter*, and *creS* mutants form straight rather than curved rods (Figure 1) (Ausmees et al., 2003). Crescentin and animal IFs share a characteristic domain topology consisting of four coiled-coil segments spaced by variable linkers. Interestingly, proteins with this characteristic organization are not found in other bacteria, plants, or fungi. Thus, if IF proteins are indeed widespread throughout the bacterial kingdom, they must have diverged from the ancestral IF. This scenario is plausible, as a yeast IF has been identified, and its domain organization differs from that of crescentin and animal IFs (van Hemert et al., 2002). Alternatively, crescentin could represent an isolated case of horizontal gene transfer from an animal to *Caulobacter*. The mechanism by which crescentin exerts its effects on *Caulobacter* cell shape remains unknown.

Bacterial Cells Are Subcellularly Organized

Cells must simultaneously regulate and coordinate multiple processes that often need to be sequestered from one another, requiring a defined subcellular architecture that localizes molecules to the right place at the right time. Cellular polarity, essential to many cell types, is a striking manifestation of this organization, and bacteria are no exception. Bacteria have long been known to possess polar structures such as flagella, pili, and stalks. With recent advances in fluorescent microscopy, it has become clear that bacteria are also polarized at the molecular level. It is perhaps no surprise that the proteins that help assemble polar structures, such as pili and flagellar components, localize to these poles. In multiple bacterial species, however, dynamic polarized protein localization has also been observed for sensory and regulatory signal transduction proteins that mediate such processes as chemotaxis, cell cycle progression, pathogenesis, and cellular differentiation (Alley et al., 1992; Maddock and Shapiro, 1993; Shapiro et al., 2002; Webb et al., 1995).

Caulobacter crescentus is a bacterium that uses polarity to regulate the progression of its cell cycle (Jensen et al., 2002), during which it divides asymmetrically to produce a larger stalked cell and a smaller swarmer cell (Figure 2A). The stalked cell is nonmotile and adheres to surfaces through an adhesive holdfast found

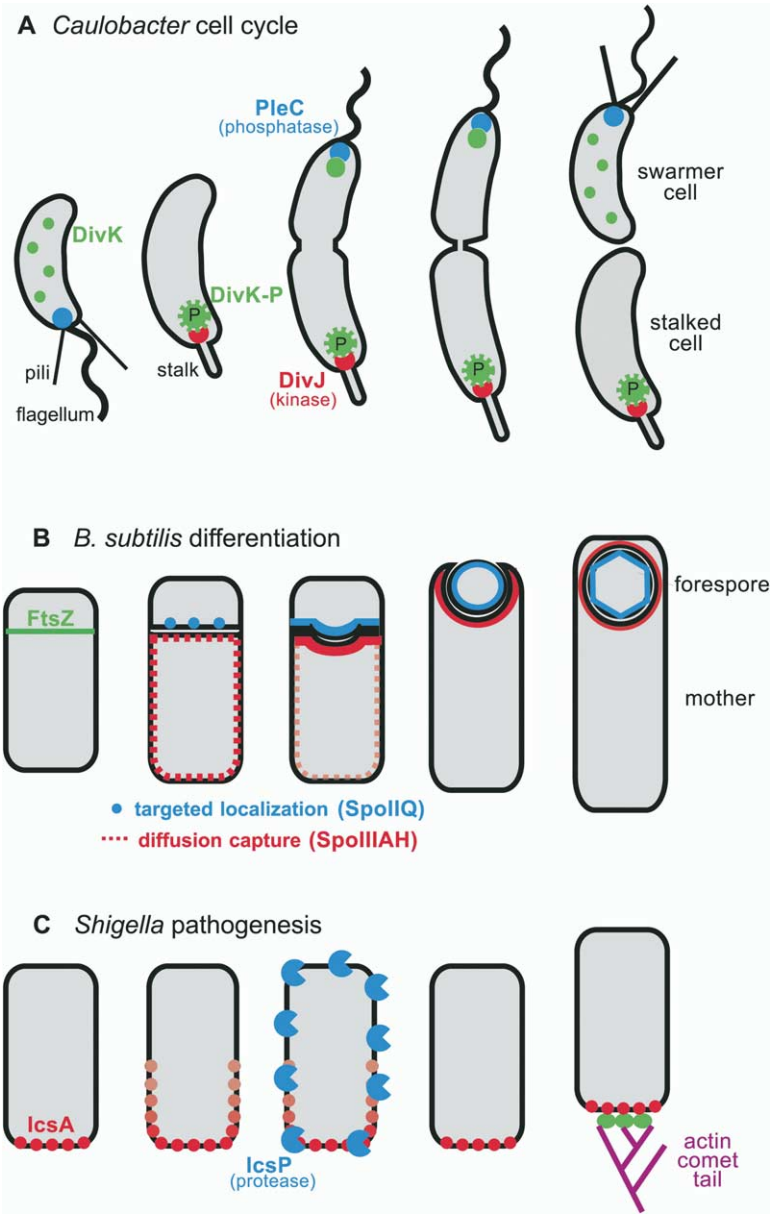

Figure 2. Bacteria Are Subcellularly Organized Cells that Use Their Organization to Regulate Their Cell Cycle, Differentiation, and Pathogenesis

(A) The asymmetric *Caulobacter* cell cycle is regulated by, among other proteins, the oppositely localized DivJ kinase (red) and PleC phosphatase (blue). PleC and DivJ cause their mutual substrate, DivK (green), to be phosphorylated (P) in the stalked cell and dephosphorylated in the swarmer cell (Matroule et al., 2004).

(B) During *B. subtilis* spore differentiation, FtsZ (green) translocates toward the cell pole, generating a polar septum. Forespore proteins such as SpoIIQ (blue) are initially targeted to the polar septal membrane, while mother cell proteins such SpoIIIAH (red) are initially dispersed throughout the mother cell. Interactions between SpoIIQ and SpoIIIAH capture and enrich SpoIIIAH at the septum and prevent SpoIIQ from diffusing away (Blaylock et al., 2004).

(C) The *Shigella* pathogenesis determinant IcsA (red) is initially localized to one pole, and a ubiquitous IcsA-specific protease (blue) maintains a sharp peak of IcsA at that pole. The polar IcsA interacts with host factors (green) to nucleate actin comet tails (purple) that propel *Shigella* around the cell (Robbins et al., 2001).

at the tip of the stalk, a cellular protrusion present at one pole of the cell. The stalked cell can be viewed as a stem cell, as it immediately reenters the cell cycle to generate another stalked cell and a differentiated swarmer cell. The swarmer cell is motile, with a flagellum and pili at one cell pole, and is quiescently arrested in the G1 phase of the cell cycle. Later, the swarmer cell sheds its flagellum and pili and grows a stalk at the same pole, differentiating into a new stalked cell. At this swarmer-to-stalked cell transition, the cell also reenters the cell cycle, initiating DNA replication.

Caulobacter's poles act as organizing centers. Multiple two-component signaling proteins such as the PleC, DivJ, and CckA histidine kinases and the CtrA, DivK, and PleD response regulators are dynamically localized to different poles at different times and are important cell cycle effectors (McGrath et al., 2004). The localization of the PleC histidine kinase/phosphatase and DivJ kinase to opposite poles is thought to differentially regulate the activity of their shared substrate, DivK, in the two daughter compartments (Figure 2A) (Matroule et al., 2004). Meanwhile, the CtrA response regulator, which serves as a master regulator of cell cycle progression, is localized to a specific pole preceding its cell cycle-regulated proteolysis (Ryan et al., 2004).

The bacterial CtrA and the eukaryotic CDK/cyclin master cell cycle regulators are both controlled by oscillating levels of transcription, proteolysis, phosphorylation, and subcellular distribution (McAdams and Shapiro, 2003). The molecular details of these regulatory processes are not conserved: CtrA is degraded by the ClpXP protease, while cyclins are degraded by ubiquitin-mediated proteosome targeting; CtrA is phosphorylated on aspartate, while cyclins are phosphorylated on

serines and threonines; and CtrA moves on and off the cell pole, while cyclins shuttle between the nucleus and cytoplasm. Nonetheless, the regulatory logic that drives the cell cycle circuits of *Caulobacter* and eukaryotes is virtually identical, suggesting that any new layers of regulation discovered in one system are likely to exist in the other.

Polar protein localization also plays a role in bacterial pathogenesis. Though *Streptococcus pyogenes* cells are morphologically symmetric spheres, they localize their bulk Sec protein secretion apparatus to a single subcellular domain, which in turn causes their pathogenic virulence factors to be asymmetrically secreted at that site (Rosch and Caparon, 2004). Other pathogens, such as *Yersinia pestis*, secrete their virulence factors through a specialized type III secretion machinery (Cheng and Schneewind, 2000). Though type III secretion can occur throughout the cell membrane, virulence factor secretion is specifically polarized to the site of contact between host cells and pathogens (Persson et al., 1995). The intracellular pathogens *Listeria monocytogenes* and *Shigella flexneri*, respectively, localize their ActA and IcsA pathogenesis determinants to one pole (Goldberg et al., 1993; Kocks et al., 1993). ActA and IcsA have domains that extend out of the bacteria and into the host cell cytoplasm, where they nucleate actin filament assembly (Figure 2C) (Cossart and Sansonetti, 2004). The actin filaments form comets that push the bacteria around the cell and generate enough force to propel these bacteria from the cytoplasm of one cell to the next, thereby avoiding the extracellular sentinels of the immune system. Polarized ActA and IcsA localization facilitates this propulsion by helping the actin comet form and push the bacterium in a single direction.

The cell poles are not the only subcellular destination for bacterial proteins. As discussed above, the localization of FtsZ to the division plane recruits a whole cascade of cell division proteins to that site. In yet another example of complex cellular dynamics, *B. subtilis* can change its entire life cycle to form hardy spores. *B. subtilis* normally divides at the cell center, but in response to various stresses, such as starvation, it shifts its division plane toward one pole (Stragier and Losick, 1996). This asymmetric cell division generates two different daughter cells: a larger mother cell and a smaller forespore (Figure 2B). The mother cell then engulfs the forespore, in a process similar to phagocytosis, and eventually lyses, releasing the mature spore. The different developmental programs executed by the mother and forespore are controlled and coordinated by a hierarchical cascade of transcription factors, but an early event in this process is the dynamic translocation of FtsZ from midcell toward a pole (Ben-Yehuda and Losick, 2002). The polar septum established by this translocated FtsZ becomes the organizing center for *B. subtilis* sporulation, serving as the localization site of the cell fate determinants, cell-cell signaling complexes, and cell engulfment proteins that regulate and execute sporulation (Errington, 2003).

Thanks to advances in fluorescence microscopy such as confocal and deconvolution microscopy, much of a sample's out-of-focus light can be either mechanically or computationally eliminated (Agard et al., 1989; Carrington et al., 1995; Fung and Theriot, 1998). Using these techniques, we can now see that proteins are not just localized to general regions of bacterial cells but can actually be assembled into complex subcellular structures. In addition to the previously discussed MreB, spiraled structures are also formed by the MinC/D/E cell division inhibitors, the SetB sugar transporter, components of the Sec secretory machinery, the LamB outer membrane protein, and, in some cases, FtsZ (Ben-Yehuda and Losick, 2002; Campo et al., 2004; Espeli et al., 2003; Gibbs et al., 2004; Shih et al., 2003). The interrelatedness of these spiraled structures remains unclear, though preliminary evidence suggests that they may be independent. Since the spiral is such a prevalent organizational geometry in bacteria, it will prove interesting to see if it is also used in eukaryotes. This notion holds promise, as the fission yeast medial ring-like lipid rafts appear spiraled in *cdc15* mutants, suggesting that other eukaryotic contractile ring components may actually consist of condensed spirals (Takeda et al., 2004). With the implementation of even higher resolution imaging techniques such as structured illumination, cryoelectron tomography, and soft X-ray microscopy, we should be able to image cells at resolutions below the diffraction limit of visible light, allowing us to investigate spirals and other organizational geometries in greater detail. Insights into protein temporal and spatial dynamics achieved through advanced fluorescence techniques such as speckle analysis, single molecule imaging, FRET, and FRAP will bolster these structural insights (Tsien, 2003).

Subcellular organization is not limited to proteins. Both eukaryotes and bacteria direct specific chromosomal regions to specific cellular locations. Bacterial chromosomes are organized such that each locus is localized to a cellular position that linearly corresponds to its genomic position. For example, in *E. coli*, *B. subtilis*, and *Caulobacter*, the origins of replication are localized toward the cell ends, the termini are localized near the cell middle, and the loci in between the origin and terminus are linearly deployed between the cell ends and middle (Niki et al., 2000; Teleman et al., 1998; Viollier et al., 2004). There are also species-specific features of chromosome organization: *E. coli* and vegetatively growing *B. subtilis* origins localize to the quarter cell positions, whereas *Caulobacter* and sporulating *B. subtilis* origins localize to the extreme poles of the cell (Jensen et al., 2002; Pogliano et al., 2003; Sherratt, 2003). Proper chromosomal architecture is important for both cell division and differentiation. Mechanisms exist to ensure that, before *E. coli* cells divide, the two replicated chromosomes are untangled and separated into the two daughters (Sherratt, 2003). *B. subtilis* cells exploit their chromosomal organization during sporulation, as their asymmetric septum traps the majority of the smaller forespore's chromosome in the larger mother cell. Before it can be translocated into the forespore, the trapped chromosome establishes a transient genetic asymmetry, during which the genes distal to the origin (including the inhibitor of forespore development, SpoIIAB) are absent from the forespore and present in two copies in the mother cell (Dworkin and Losick, 2001).

Besides reproducibly localizing proteins and DNA,

bacteria may also localize RNA molecules, lipids, and in some species even subcellular organelles. RNA localization has been proposed to facilitate the secretion of flagellar proteins (Aldridge and Hughes, 2001). In addition, the outer membrane lipid composition of *E. coli* cell poles differs from that of the rest of the cell (Mileykovskaya and Dowhan, 2000), potentially acting as a bacterial analog of eukaryotic membrane microdomains. Remarkably, some bacteria, such as *Magnetospirillum*, possess membrane bound organelles that are subcellularly localized (Komeili et al., 2004). Thus, even though bacteria are tiny and lack membrane bound organelles, they are highly organized and heterogeneous, sequestering different molecular complexes and functions to different cellular domains.

Several Mechanisms Underlie Bacterial Subcellular Organization

The mechanisms by which proteins are localized are under intense investigation in both prokaryotes and eukaryotes and can generally be explained by one of two mechanisms: diffusion capture or targeted localization. Examples of each of these mechanisms have been documented in bacteria, and *B. subtilis* uses both strategies to localize proteins to the polar septum of sporulating cells. Transmembrane proteins are directly targeted to the forespore septal membrane but are initially randomly distributed throughout the mother cell (Figure 2B) (Rubio and Pogliano, 2004; Rudner et al., 2002). Interactions between the extracellular domains of forespore and mother cell proteins serve to both capture mother cell proteins at the septum and prevent forespore proteins from diffusing away from the septum (Blaylock et al., 2004). In this fashion, the interacting surfaces of two cells are exploited to localize proteins in both compartments. Illustrating a different strategy, the *Shigella* IcsA protein is initially inserted at one cell pole, and a uniformly distributed IcsA-specific protease maintains a sharp peak of IcsA at that pole (Figure 2C) (Robbins et al., 2001).

In eukaryotes, targeted protein delivery is often achieved by the transport of proteins along ordered arrays of polarized cytoskeletal filaments such as yeast actin cables or neuronal microtubule bundles. In *Caulobacter*, the MreB actin homolog is required for the dynamic localization of cell cycle regulatory proteins to the correct cell pole (Gitai et al., 2004). Surprisingly, cells that have lost and then regained MreB do not retain memory of their polarization prior to depletion, localizing proteins to random poles (Gitai et al., 2004). Thus, MreB is not just permissively required for polar localization but also contains the polarity information to direct proteins to specific poles. Akin to eukaryotic cytoskeletal trafficking, the molecular polarity inherent in an MreB actin-like filament may thus be translated into a mechanism for directing global cell polarity.

Though diffusion capture and targeted localization may explain much of protein distribution, both mechanisms depend on the existence of previously localized determinants. Diffusion capture requires a separate mechanism to localize the capturing factor, while targeted localization requires a mechanism to structure or direct the targeting machinery as well as yet another mechanism to keep the localized factor in the correct

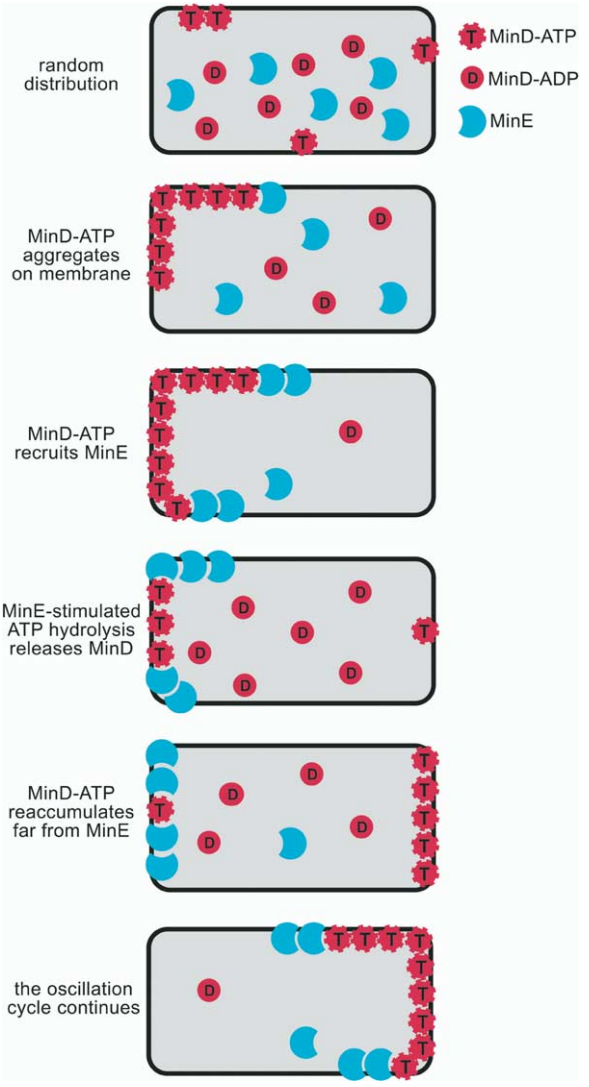

Figure 3. A Self-Organizing System for Finding a Cell's Center

In *E. coli*, FtsZ polymerization is directed to the cell's center by a rapid pole-to-pole oscillation of MinC, an FtsZ polymerization inhibitor whose time-averaged lowest concentration is at the center. MinC oscillates by associating with the MinD ATPase (red). MinD-ADP (D) is cytoplasmic, while MinD-ATP (T) binds the membrane, polymerizes in a cooperative membrane, and eventually recruits MinE (blue) to the membrane. MinE activates the conversion of MinD-ATP to MinD-ADP, causing it to dissociate from the membrane. In the cytoplasm, ADP is exchanged for ATP, and this new MinD-ATP begins to reassemble at the point of the cell furthest from MinE, namely the opposite pole. Thus, the interactions of MinD and MinE are sufficient to drive their sustained oscillation along the long axis of the cell.

place. Most protein localization studies thus only serve to push the question back one level rather than to actually address the origin of subcellular organization. One exception is the self-organizing Min system for FtsZ localization in *E. coli* (Figure 3).

The medial placement of the FtsZ ring in *E. coli* is regulated by the Min proteins. FtsZ polymerization is inhibited by MinC, which also binds to the MinD ATPase (Hu and Lutkenhaus, 2000; Hu et al., 1999). MinC and

MinD rapidly oscillate from pole to pole, such that the time-averaged concentration of MinC is lowest at the cell middle, thereby directing FtsZ polymerization to this location (Hu and Lutkenhaus, 1999; Raskin and de Boer, 1999a; Raskin and de Boer, 1999b). MinD drives this oscillation by cooperatively polymerizing and binding the membrane in its ATP bound state and then recruiting MinE (Hu et al., 2002; Lackner et al., 2003). MinE activates MinD-ATP hydrolysis, thereby sweeping MinD off the membrane. Later, the cytoplasmic MinD exchanges its ADP for ATP and reassembles at the point in the cell furthest from the MinE aggregate, namely the opposite cell pole. Mathematical modeling has demonstrated that these few interactions are sufficient to self organize; given a defined container and starting with randomly dispersed MinC/D/E and FtsZ, the Min proteins will find the long axis of the cell, oscillate along it, and drive FtsZ polymerization to its center (Figure 3) (Howard et al., 2001; Huang et al., 2003; Kruse, 2002; Meinhardt and de Boer, 2001). This system could also generate cell polarity: since cell division causes the mother midcell to become the new poles of the two daughters, the midcell-localized FtsZ could recruit a factor whose localization would persist after division and specifically mark one cell pole. Such a marker could in turn recruit additional factors to propagate polarity. These insights represent a breakthrough that provides, to my knowledge, the first and only description of a system that can explain a specific subcellular localization pattern without invoking any previously localized determinants.

The reproducible and dynamic organization of bacterial chromosomes demonstrates that bacteria also possess mechanisms for localizing DNA, and we are accumulating an ever-growing list of proteins that affect this process. These proteins include replication factors (e.g., replisome), DNA compaction proteins (topoisomerases, SMC, MukB, and the histone-like HU/H-NS), cytoskeletal elements (MreB), putative partitioning proteins (ParA and ParB), dimer resolution proteins (XerCD), crossmembrane translocation pumps (FtsK/SpoIIIE), and anchoring factors (RacA) (Sherratt, 2003). The interrelatedness of chromosomal positioning, movement, and replication has made it difficult to dissect the specific roles of many of these proteins. One solution to this problem came from the characterization of a small compound, A22, that can rapidly and directly perturb MreB (Gitai et al., 2005). This type of pharmacological approach enables the temporal dissection of MreB's multiple cellular functions and has already been combined with biochemical experiments to demonstrate a direct role for MreB in trafficking a specific region of the *Caulobacter* chromosome from one pole to the other (Gitai et al., 2005). Coupled with the development of techniques to visualize chromosome segregation in vivo and the identification of *cis*-acting centromeric elements (Lau et al., 2003; Yamaichi and Niki, 2004), these studies promise significant advances in our understanding of bacterial mitosis in the near future.

Bacterial Multicellularity

In this review, I have focused on the emergence of bacteria as viable models for studying the unicellular processes of cytoskeletal dynamics, subcellular organization, and cell division. However, it is worth noting that most bacteria are actually community-oriented organisms that communicate with each other and develop into multicellular structures. Indeed, communal living is now considered the default growth state for wild bacteria (Shapiro, 1998). Bacteria could thus serve as models for understanding long- and short-range intercellular signaling, morphogens, cell adhesion, and tissue formation. Since these processes have been thoroughly covered in several recent reviews (Bassler, 2002; Brun and Shimkets, 2000; Kaiser, 2001; Webb et al., 2003), I will only highlight a few examples.

Through an elegant process of lateral inhibition, the cyanobacterium *Anabaena* responds to low nitrogen levels by directing a few individual cells to develop into nitrogen-fixing heterocysts. Early in their development, heterocysts secrete a small polypeptide that prevents the overproduction of heterocysts by inhibiting the differentiation of neighboring cells (Golden and Yoon, 2003). Other bacteria, like *Myxococcus xanthus*, use an elaborate series of different signals to respond to stressful environmental conditions by swarming together to generate intricate and specialized fruiting bodies (Kaiser, 2004). Spores develop inside the fruiting body and are released when conditions improve.

Larger bacterial communities are also capable of communicating with each other to coordinate their activities. Through a process known as quorum sensing, bacteria constantly monitor their density by secreting and sensing both species-specific and interspecies cues. With this information, bacteria coordinate the initiation of programs whose success requires a large population. For example, a small number of pathogenic *E. coli* would not be able to colonize an intestine, so they do not express their virulence genes until a critical concentration of *E. coli* is present (Sperandio et al., 1999). Quorum sensing also regulates other community activities such as biofilm formation, in which large groups of bacteria adhere to a surface, aggregate, and surround themselves with a thick extracellular coat (Stanley and Lazazzera, 2004). Biofilms are themselves interesting examples of bacterial development, as they grow into complex multicellular structures with intricate channels for nutrient delivery and waste removal (O'Toole et al., 2000). Biofilm development has great clinical relevance, as bacteria in biofilms are highly resistant to antibiotic treatment due to their slimy coat and elaborate irrigation systems. Thus, like much of bacterial cell biology, understanding biofilm development is of both conceptual and practical importance.

Conclusion

Though bacterial cell biology is still an emerging field, it is now clear that, despite their diminutive size, bacteria are highly organized and dynamic cells. The realization that bacteria can serve as both molecular and conceptual models has caused us to reassess the traditional views of both bacteria and the nature of model systems. While the emergence of bacteria as model cells is a significant advance, the study of bacterial cell biology is also important in its own right. Bacteria are wondrously diverse and resourceful, occupying virtually every environmental niche imaginable. Understanding these cells should aid developments in fields

such as agriculture, bioremediation, and energy production. In addition, bacteria may hold the key to understanding bacterially derived plant and animal organelles such as mitochondria and chloroplasts. Finally, bacterial cell biology may prove to be of great clinical importance in combating infectious diseases. The molecular differences between prokaryotic and eukaryotic cells could be exploited to identify a new generation of antimicrobial drug targets to replenish our critically depleted clinical arsenal.

As we continue to learn about the molecular underpinnings of both bacterial and eukaryotic cell biology, a number of themes continuously emerge. These themes include the centrality of the cytoskeleton in regulating and executing key cellular processes, the organized and dynamic nature of the subcellular architecture that reproducibly positions proteins and chromosomal regions to the right place at the right time, and the capacity of cells to communicate with each other to coordinate multicellular events. Since bacteria accomplish many of the same cellular tasks as eukaryotes, work in bacteria is already paying dividends with the dissection of processes whose eukaryotic counterparts have been elusive, such as mechanisms for self-organizing topology. The synergistic power of studying multiple systems with different advantages should continue to advance our understanding of the biology of all cells.

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