

Tiny cells meet big questions: a closer look at bacterial cell biology

Erin D. Goley

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

ABSTRACT While studying actin assembly as a graduate student with Matt Welch at the University of California at Berkeley, my interest was piqued by reports of surprising observations in bacteria: the identification of numerous cytoskeletal proteins, actin homologues fulfilling spindle-like functions, and even the presence of membrane-bound organelles. Curiosity about these phenomena drew me to Lucy Shapiro's lab at Stanford University for my postdoctoral research. In the Shapiro lab, and now in my lab at Johns Hopkins, I have focused on investigating the mechanisms of bacterial cytokinesis. Spending time as both a eukaryotic cell biologist and a bacterial cell biologist has convinced me that bacterial cells present the same questions as eukaryotic cells: How are chromosomes organized and accurately segregated? How is force generated for cytokinesis? How is polarity established? How are signals transduced within and between cells? These problems are conceptually similar between eukaryotes and bacteria, although their solutions can differ significantly in specifics. In this Perspective, I provide a broad view of cell biological phenomena in bacteria, the technical challenges facing those of us who peer into bacterial cells, and areas of common ground as research in eukaryotic and bacterial cell biology moves forward.

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EARLY SIGNS OF ORGANIZATION

By now, it is widely recognized that a description of bacteria as "bags of enzymes" that lack dedicated mechanisms of spatial order is inaccurate and obsolete. Early indications that some bacterial species might possess mechanisms of spatial organization came from simply observing cell morphology: sporulating organisms like *Bacillus subtilis* develop a forespore at one end of the cell and not the other; dimorphic species like *Caulobacter crescentus* exhibit distinct polar appendages at different stages of the cell cycle; more broadly, bacteria can adopt a variety of cell shapes that result from polarized growth (Young, 2006). Each of these examples suggests an underlying architecture to spatially restrict growth, signaling, or development. Indeed, with the advent of green fluorescent protein (GFP) fusion technology in the mid-1990s, many bacterial proteins were demonstrated to localize within the cell, often

dynamically so in response to cell cycle or developmental cues. Some of the earliest studies with GFP fusion proteins illustrated dynamic localization of sporulation factors (Webb et al., 1995), cell cycle regulators (Jacobs et al., 1999), and cell division proteins (Ma et al., 1996). Since then, hundreds of bacterial proteins have been localized, and a recent whole-genome view of protein localization in *C. crescentus* indicates that >10% of proteins have a particular address within the cell (Werner et al., 2009). Beyond proteins, the bacterial chromosome is highly ordered in space (Reyes-Lamothe et al., 2012), mRNAs can be localized (Montero Llopis et al., 2010), lipid microdomains have been observed (Mileykovskaya and Dowhan, 2000), and small signaling molecules can show asymmetric distribution (Christen et al., 2010). Precise spatial distribution of proteins and other cellular components appears to be a universal feature of bacteria, even those that lack obvious asymmetry.

UNEXPECTED LIKENESSES

Perhaps it is not so surprising that bacteria localize their proteins and other biomolecules. More startling are discoveries that defy conventional definitions of "eukaryote" and "prokaryote." For example, I was taught in undergraduate cell biology that endomembrane systems were a defining characteristic of eukaryotic cells. Although it is certainly true that bacteria do not possess the stereotypical organelles of eukaryotes, there are organisms that come surprisingly

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Address correspondence to: Erin D. Goley (egoley1@jhmi.edu).

Abbreviations used: ECT, electron cryotomography; GFP, green fluorescent protein.

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close. These bacterial species elaborate extensions of their cytoplasmic membranes to create specialized compartments or even form structurally independent membrane-bound organelles. The magnetosomes of *Magnetospirillum magneticum* (Komeili et al., 2006), photosynthetic membranes of several classes of bacteria (Murat et al., 2010), and nuclear envelope (Fuerst and Webb, 1991) and endocytosis events (Lonhienne et al., 2010) in planctomycetes like *Gemmata obscuriglobus* are emerging as models for understanding bacterial endomembranes. A focus on the cell biology of these compartments is fairly recent, and there are more questions than answers. How are internal membranes shaped in bacteria? How are they duplicated and segregated as the cell grows and divides? How are proteins and other molecules targeted to and retained in these compartments? Answering these questions may provide insight into the origins of eukaryotic organelles and allow engineering or manipulation of compartments for synthetic biology applications.

A second example of a canonically eukaryotic element popping up in bacteria, and the one that first drew my attention to bacterial cell biology, was the discovery of bacterial cytoskeletal proteins. FtsZ was the first such protein described: in the early 1990s it was shown to be a polymerizing GTPase that localizes to the cell division site (Erickson, 1995), and in 1998 it was confirmed as a tubulin homologue when high-resolution structures of each revealed the remarkable similarity of their folds (Löwe and Amos, 1998; Nogales et al., 1998). Since then, the list of bacterial cytoskeletal proteins has expanded to include dozens of families of actin homologues, at least four families of tubulin homologues, intermediate filament-like proteins, CTP synthase, and bacterial-specific, polymer-forming proteins like Walker A cytoskeletal ATPases and bactofilins (Ingerson-Mahar and Gitai, 2012). Bacterial cytoskeletal proteins fulfill cellular roles analogous to their eukaryotic counterparts: segregating genetic material, directing polarity and growth, orchestrating cytokinesis, organizing membranes, and scaffolding protein complexes. We are making significant strides in the biochemical and structural characterization of these factors, but progress in understanding their regulation and mechanisms of action in the cell has been slower. Do FtsZ and the actin homologue MreB generate force to direct cell wall remodeling during division and growth or simply serve as scaffolds for the protein complexes that do the heavy lifting? What polymeric species are present in the cell, and how do polymer structures relate to their function? The bacterial cytoskeleton has drawn substantial interest from eukaryotic cell biologists, as findings about the behavior, structure, and regulation of bacterial actins and tubulins may be relevant to their eukaryotic homologues and may establish unifying principles of cytoskeletal function (Löwe and Amos, 2009).

Bacterial species encompass enormous diversity, so I cannot begin to enumerate all of the cell biological questions they present. In the foregoing I described two areas that are surprisingly reminiscent of canonical eukaryotic cell biology. In looking through the program from the latest ASCB Annual Meeting, I can think of bacterial cell biological processes that could fall readily under most of the headings for Symposia, Minisymposia, and Poster Sessions. Table 1 provides examples of parallels to major areas of eukaryotic cell biological research and directs the reader to reviews on these topics.

THE UPS AND DOWNS OF WORKING WITH BUGS

My move from studying actin regulation in eukaryotic systems to studying FtsZ function in a bacterium revealed advantages, and challenges, to working on bacterial cell biology. An obvious

disadvantage is the fact that most bacteria are on the same size scale as eukaryotic organelles, making imaging subcellular structures a challenge. When the diameter of your cell is only twice the resolution limit of a light microscope, every incremental increase in resolution counts. With superresolution imaging techniques the picture we obtain of the bacterial cytokinetic ring in living cells, for example, is vastly improved over widefield fluorescence microscopy (Fu et al., 2010; Biteen et al., 2012; Strauss et al., 2012), but it is not yet to the point that we can resolve the individual FtsZ filament structures within it. Electron cryotomography (ECT) allows for beautiful visualization of cytoskeletal filaments, membranes, and other macromolecular structures at high resolution in cryopreserved cells (Li and Jensen, 2009). ECT relies on natural contrast in the sample, however, and is not yet compatible with protein-labeling strategies, so the picture it presents may not be comprehensive. Bacterial cell biologists are poised to benefit in a big way from continued technical advances in superresolution, single-molecule, and ECT approaches. We would also benefit from the development of tools that allow us to specifically and rapidly dissect cell biological phenomena. I sorely miss the array of well-characterized small-molecule inhibitors and probes of cytoskeletal, trafficking, or other events available for eukaryotic cells. Here, again, it is a matter of time, as continued effort is being invested in developing the tools we need to characterize cell biological events in bacteria.

Along with the downsides, there are the tremendous advantages to working in bacteria: they have comparatively small genomes, short reproduction times, and, in many cases, well-developed genetic and genomic tools. Thus it is fairly fast and straightforward to address the function of a gene of interest in vivo or take a systems-level view of a particular phenomenon. The relatively small parts list of bacterial cells makes it feasible to address fundamental cell biological questions comprehensively (e.g., Skerker et al., 2005; McKenney et al., 2010; Goley et al., 2011), reconstitute them in vitro (e.g., Garner et al., 2007; Loose et al., 2008), and model them in silico (e.g., Huang et al., 2003; Shen et al., 2008). Bacteria also offer tremendous ecological, morphological, and phylogenetic diversity that can be harnessed to select the ideal model system for a question of interest. For example, progress in understanding the structural basis of bacterial cytoskeletal function has been rapid in many cases, in part owing to the ability to use homologues from thermophilic bacteria for crystallographic studies. Moreover, as in eukaryotes, the ability to address the same cellular process in phylogenetically distant organisms allows one to discern universalities and variations in the execution and regulation of an event of interest. This approach has revealed that bacteria, perhaps more often than eukaryotes, can evolve distinct mechanisms for controlling the same fundamental process. A mitotic spindle-like chromosome segregation system (ParABS) exists in organisms like *C. crescentus*, *Vibrio cholerae*, and sporulating *B. subtilis*, for example, each of which exhibits clear cell polarity and targets its origin of replication to the cell pole. No such dedicated machinery has been identified in the morphologically symmetric organism *Escherichia coli*, which localizes its origin of replication near midcell (Reyes-Lamothe et al., 2012). These differences highlight the inventiveness and efficiency of bacterial cells in optimizing their cell biology to their lifestyle and suggest that we might capitalize on these molecular distinctions to develop antibiotics that are specific to a subset of bacteria. Finally, what drew me to the field of bacterial cell biology is the fact that it is in its infancy and is laden with exciting, fundamental questions. Not only do we have work to do in uncovering the mechanistic basis of cell biological events and the nuances of their regulation in diverse species, but in

Cell biological phenomenon	Examples of model systems	Relevant reviews
Spatial control of growth, division, and morphology	<i>Escherichia coli</i> , <i>Bacillus subtilis</i> , <i>Caulobacter crescentus</i> , <i>Streptococcus pneumoniae</i>	Typas et al. (2012), Young (2010)
Chromosome and plasmid organization and segregation	<i>B. subtilis</i> , <i>C. crescentus</i> , <i>E. coli</i> , <i>Vibrio cholerae</i> , <i>Streptomyces coelicolor</i>	Reyes-Lamothe et al. (2012)
Membrane-bound organelle formation and function	<i>Gemmata obscuriglobus</i> , <i>Magnetospirillum magneticum</i> , <i>Rhodopseudomonas palustris</i>	Fuerst and Sagulenko (2011), Murat et al. (2010)
Cytoskeletal structure and function	<i>B. subtilis</i> , <i>C. crescentus</i> , <i>E. coli</i> , <i>Thermotoga maritima</i> (structure)	Ingerson-Mahar and Gitai (2012), Löwe and Amos (2009), Wickstead and Gull (2011)
Cell cycle regulation	<i>C. crescentus</i> , <i>B. subtilis</i> , <i>E. coli</i>	Curtis and Brun (2010), Haeusser and Levin (2008)
Cell polarity	<i>C. crescentus</i> , <i>B. subtilis</i> , <i>Streptomyces</i> species	Dworkin (2009)
Cell motility	<i>B. subtilis</i> , <i>E. coli</i> , <i>Myxococcus xanthus</i> , <i>Proteus mirabilis</i>	Patrick and Kearns (2012), Zhang et al. (2012)
Development	<i>B. subtilis</i> , <i>C. crescentus</i> , <i>M. xanthus</i>	Curtis and Brun (2010), McKenney et al. (2012), Zhang et al. (2012)
Multicellularity and intercellular communication	<i>B. subtilis</i> , <i>Pseudomonas aeruginosa</i> , <i>M. xanthus</i> , <i>Vibrio</i> species	Aguilar et al. (2007), Ng and Bassler (2009), Zhang et al. (2012)

The listed model systems and reviews are intended to be a starting point for more information and are by no means comprehensive.

TABLE 1: Bacterial parallels to eukaryotic cell biological phenomena and examples of the model systems used to study them.

addition there are undoubtedly phenomena that remain to be described and, even in well-studied processes like cytokinesis or chromosome organization, basic mechanisms to be elucidated.

THE COMMON GROUND

So why should a eukaryotic cell biologist care about what is going on in bacterial cells? When it comes down to it, eukaryotic and bacterial cells are made of the same biomolecules, obey the same rules of chemistry and physics, and, therefore, often come up with similar strategies for accomplishing the same goal. In learning about a new phenomenon in bacteria, I am often struck by similarity to a cell biological process in eukaryotes and vice versa. Recently a diffusion barrier was described that limits entry of periplasmic and membrane proteins to the polar stalk of *C. crescentus* (Schlimpert et al., 2012). This discovery immediately made me think of the diffusion barriers at the base of primary cilia (Hu and Nelson, 2011). Although the molecules doing the job are different, the biophysical basis and functions of these barriers may be the same. Similarly, bacterial cells face challenges similar to those of fungal or plant cells during cytokinesis: each requires coordination of membrane ingression with cell wall remodeling in the face of high internal turgor pressure. Understanding how one of these systems works illuminates potential mechanisms in the others. From a practical perspective, new technologies for imaging, automated image and data analysis, or high-throughput studies advanced by bacterial cell biologists will benefit eukaryotic cell biologists and vice versa.

Research in bacterial cell biology is motivated by a variety of factors, ranging from the applied (e.g., identifying and characterizing targets for antibiotics) to the fundamental (e.g., elucidating basic biophysical or biochemical mechanisms behind polarity establishment or chromosome organization). With growing awareness of the microbiome, growing interest in synthetic biology, and growing resistance of bacterial pathogens to existing antibiotics, the practical benefits of a detailed knowledge of bacterial cells continue to expand. Bacterial cell biologists are using all of the tools of modern

biology—genetics, genomics, microscopy, biochemistry, systems biology, biophysics, and so on—to uncover the mechanisms and meaning of cell biological phenomena. Through this Perspective, I hope to have convinced eukaryotic cell biologists that it is worth their time to check out that talk on bacterial development or signaling or cytokinesis when they see it on the seminar schedule or in the ASCB Annual Meeting program. Likewise, I urge bacterial cell biologists to attend ASCB meetings and other conferences traditionally aimed at eukaryotic cell biologists. Once they get past the obvious differences, they may just find familiarity and inspiration.

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