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GUEST COMMENTARY

Changing Views on the Nature of the Bacterial Cell: from Biochemistry to Cytology†

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When the authors were graduate students in the 1960s, the bacterial cell was generally viewed as an amorphous vessel housing a homogeneous solution of proteins. Trained as biochemists, we would break cells open, subject the disrupted cells to centrifugation, and separate the supernatant fluid from the membranous material that collected at the bottom of the centrifuge tube. Some enzymes, such as the replicase for the RNA phage F2, the subject of thesis research by L.S., were to be found in the supernatant fluid. Other enzymes, such as those involved in the biosynthesis of the O-antigen component of the lipopolysaccharide of *Salmonella*, the subject of doctoral work by R.L., were associated with the membrane. No differentiation was imagined other than the distinction between membrane proteins and cytoplasmic proteins. This is a view that persisted for a surprisingly long time. Of course, some bacteria have conspicuous proteinaceous appendages, such as flagella and pili, and some bacteria, such as *Caulobacter crescentus*, are conspicuously asymmetric. But only in this decade, and chiefly over the last few years, has it become apparent that cytoplasmic and membrane proteins can, and often do, have particular subcellular addresses, that these addresses can change over time, sometimes with extraordinary rapidity, and that an understanding of function requires knowledge not only of what a protein does but often of where it is in the cell.

A landmark in cytological studies of bacteria was the demonstration in 1991 (5) that the cell division protein FtsZ assembles into a ring-like structure known as the Z ring at the site of cell division. This was significant because it demonstrated that a cytoplasmic protein could localize to a particular site in the cell and that its location was pertinent to its role in cytokinesis. Complementing and extending the demonstration of the localization of a cytoplasmic protein was the discovery (3, 22) that transmembrane chemoreceptors in *Escherichia coli* and *Caulobacter* are localized to the cell poles and that the cytoplasmic proteins CheA and CheW, which interact with the chemoreceptors, are found at the poles only when chemoreceptors are present in the cell. This showed that the cytoplasmic membrane is not uniform, that protein complexes can localize to particular regions of the membrane, and therefore that the bacterial cell is not housed in an amorphous vessel. Other early examples of protein subcellular localization were the discoveries that certain morphogenetic proteins assemble into shell-like structures during sporulation in *Bacillus subtilis* (7) and that proteins involved in nucleating actin polymerization (11, 17) are asymmetrically distributed on the cell surface of the pathogens *Shigella flexneri* and *Listeria monocytogenes*. Initially, these cytological discoveries were carried out by immunoelectron microscopy, but the introduction of immunofluorescence microscopy, initially for *E. coli* (22) and *Shigella* (10) and then for *B. subtilis* (27), greatly increased the sensitivity with which protein localization studies could be carried out, while the use of the green fluorescent protein (GFP) in bacteria (4, 15, 21, 32) has made it possible to visualize the position and movement of proteins in living cells.

Where a protein is in a cell is often crucial for understanding what it does. The assembly of FtsZ into Z rings is responsible for recruiting other (septasome) proteins involved in cytokinesis, and the site of this assembly process dictates the subsequent placement of the division septum (1, 8, 13, 21, 31, 35). In growing cells, Z-ring formation at the midcell position is responsible for binary fission whereas the switch in the site of Z-ring formation from the cell's middle to near the cell's poles underlies the process of asymmetric division that takes place during sporulation in *B. subtilis* (19). Likewise, knowing that SpoIVA assembles into a shell-like structure around the developing forespore in *B. subtilis* immediately explained the role of this morphogenetic protein in the recruitment of coat proteins to the outer surface of the maturing spore (28). Finally, the discovery that the regulatory phosphatase SpoIIE localizes to the polar septum provided a link in the chain of events from asymmetric division to the activation of sporulation genes (4).

We come to depend on knowing not only where proteins are but also where and how their positions change over time. An example of a protein that changes its subcellular address is the proprotein precursor to the sporulation transcription factor σ^E (14, 16). Pro-σ^E is a membrane-associated protein, and in the predivisional sporangium it colocalizes with the cytoplasmic membrane. During asymmetric division, however, it is redeployed to the polar septum, where the protease that is responsible for its processing is located. Finally, after proteolytic cleavage, the mature transcription factor is released into the cytoplasm, where it associates with RNA polymerase. Thus, during its activation, this transcription factor successively exhibits three subcellular addresses. Recently, it has become possible to capture movement of a membrane protein by time-lapse microscopy. A newly discovered kinase, CckA, that plays a crucial role in the cell cycle of *C. crescentus*, oscillates between deployment in the membrane all around the cell and clustering at the cell poles, a movement that occurs on a time scale of tens of minutes, representing less than 1/10th of the cell cycle (15). A working model is that the kinase is active only when sequestered at the polar location. Progression through the cell cycle, therefore, would be dictated by the migration of

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a protein from one location to another. Thus, in understanding bacterial regulatory mechanisms, we must consider the dynamic movement in three-dimensional space of proteins and protein complexes. (See the cover of this issue for time-lapse images of three examples of dynamic movement drawn from this Commentary.)

Sometimes movement of proteins can be quite rapid. Late in the cell cycle in *E. coli*, FtsZ can be seen to redeploy in less than a minute from the division septum to future sites of cytokinesis (2, 30). An extraordinary example of protein movement is provided by the behavior of the cell division inhibitor MinD in *E. coli*. MinD prevents cytokinesis from occurring at potential division sites near the cell poles. It now emerges that MinD preferentially localizes near the cell poles, but it does so in an oscillating manner, clustering first near one cell pole and then the other, with this alternation occurring with a frequency of the order of tens of seconds (29)! Not only do we need to know where proteins are but in some cases we may need to follow their movements over short increments of time.

The application of the tools of cytology has also changed our view of the organization of the bacterial chromosome and the mechanism by which it is segregated during the cell cycle. The use of fluorescence in situ hybridization (FISH) has made it possible to visualize specific sites on the chromosome in fixed cells (24), and the use of GFP fusions has made it possible to see the location of particular chromosomal regions in living cells and to follow their movement over time. GFP has been fused to proteins that naturally bind to the origin region of the chromosome (9, 20, 23). Also, a fusion of GFP to the LacI repressor has been used to decorate fluorescently a variety of sites in the chromosome into which tandem copies of the *lacO* operator have been inserted (12, 34). Work of this kind has led to the discovery that during the cell cycle the newly duplicated origins of replication move towards opposite poles of the cell (just the opposite to what had been anticipated in the replicon model). Indeed, this movement can be captured by the use of time-lapse microscopy (12, 33). Meanwhile, the complex of replication proteins seems to be held relatively stationary in the central region of the cell, a discovery that gives rise to the view that proteins involved in DNA replication constitute a kind of stationary machine through which the chromosome is spooled (18). Finally, certain plasmids, such as F and P1, have a pattern of subcellular localization of their own, initially localizing to the cell center and then after duplication rapidly moving to the quarter points in the cell (12, 25).

Our ability to visualize the organization of the bacterial cell is not limited to proteins and nucleic acids. The use of vital membrane stains, such as FM4-64 and FM1-43, makes it possible to visualize lipid bilayers in living bacteria (26, 30). In a dramatic application of these stains, Pogliano and coworkers have visualized the process of engulfment in *B. subtilis* by carrying out time-lapse microscopy during the course of sporulation (26). Their images reveal the dynamic nature of this phagocytic-like process in which the mother cell membrane migrates around, surrounds, and eventually wholly engulfs the forespore.

Finally, we comment on the impact of deconvolution microscopy on bacterial cytology (21, 26, 30). Efforts to visualize the organization of the procaryotic cell by light microscopy face the formidable challenge of the small size of bacteria, which are often only 1 to 3 μm in length. In deconvolution microscopy, a series of optical sections are collected along the Z axis by advancing the specimen relative to the objective (6). The images are then processed with a deconvolution algorithm, which removes out-of-focus light and reassigns it to its correct points of origin. This greatly improves resolution and even makes it possible to visualize structures in three dimensions by stacking deconvolved optical sections on top of one another. For example, deconvolution has been used to visualize the three-dimensional nature of Z rings in *E. coli* (30) and the assembly of a morphogenetic protein in *B. subtilis* into a shell-like structure (28).

How profoundly our view of the bacterial cell has changed since we first started our lifelong fascination with life's smallest creatures. Who would have imagined that bacteria have proteins that assemble into rings, that cluster at the poles of cells, that localize and delocalize as a function of the cell cycle, or that bounce off the ends of the cell with a periodicity of tens of seconds? Who would have suspected that the origins of replication move to the poles of cells, that the machinery for replicating DNA is stationary, and that it is the chromosome that moves through the chromosome-duplicating factory or that plasmids would jump from the cell center or the cell quarter points following their replication? The pace at which cytology is revealing the unexpected is quickening, and one wonders with anticipation what other delightful surprises await those who use the light microscope to peer inside the bacterial cell.

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