Bacterial morphogenesis: learning how cells make cells
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Bacteria furnish tractable models for complex biological processes, and morphogenesis is now taking its turn. We can already explain in general terms how such elementary forms as rods and cocci are produced, and the shapes of several individual organisms are coming into focus. In most bacteria shape is maintained by the cell wall, specifically the peptidoglycan layer, which has the attributes of a strong stiff fabric. Compliance of that fabric with turgor pressure is an important aspect of morphogenesis. The shape of the wall sacculus is determined by the way it is deposited, which is controlled by a cytoskeleton made up of two molecular families. One, related to the eukaryotic tubulins, is responsible for the construction of the septum and the poles. The other, related to eukaryotic actins, localizes peptidoglycan synthesis in the lateral walls of rod-shaped cells. Just how the cytoskeleton itself is organized remains to be discovered, but it seems likely that, as in eukaryotes, the cytoskeleton is produced by self-organized assembly, guided by the fabric of the cell.

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Bacterial morphogenesis, I maintain, is not primarily a matter of genetics, biochemistry or molecular biology (though these disciplines all have important contributions to make); to understand how organisms shape themselves, the problem must be approached from the viewpoint of physiology, the science of complex systems. The forms we encounter in the bacterial world span the range of sizes from molecules to biofilms, nanometers to centimeters. Every one represents a system of significant complexity; all can be studied by molecular methods, but only the simplest can be understood in molecular terms alone. At the present time, we cannot fully explain how any one of the ‘higher’ microbial forms is produced, nor what role it plays in the life cycle of the organism. And yet, no one who has tracked this subject for some time (more than two decades in my case, refs [1,2,3]) can fail to be impressed by the progress that has been made. Bacterial morphogenesis is at last drawing attention, and rightly so, for bacteria illustrate all the mysteries of biological complexity at the simplest and most tractable level.

The technical articles that follow document what we have learned about the genesis of particular bacterial forms and their biological meaning. As a spectator of this pursuit rather than an active participant, I shall leave the primary literature and the latest discoveries to my colleagues. My task is to trace the conceptual journey, to prize understanding over information, and to put my good eye to the end of the telescope.

Not by self-assembly
They really should have given Heinz Fraenkel-Conrat the Nobel Prize for his discovery, over fifty years ago, that purified RNA and capsid protein of tobacco mosaic virus associate spontaneously to reconstitute normal, viable and infectious virus particles. Many more instances of molecular self-assembly have been reported since, including bacteriophages, ribosomes, lipid bilayer membranes, microtubules and microfilaments, gas vacuoles and S-layers. Others will undoubtedly turn up among the ‘hyperstructures’ of bacterial cytoplasm [4]. The hallmark of molecular self-assembly, at least in principle, is that it requires no input of either energy or information beyond that contributed by the subunits. A more sophisticated variant, designated dynamic self-assembly or self-construction [3**, is illustrated by the mitotic spindle. This is a dynamic structure whose assembly does require energy consumption but no external instructions; the bacterial cytoskeleton is sure to supply further examples. Many bacterial organelles arise by stepwise assembly of some kind, as illustrated by the flagellum [5].
Well then, can we consider a bacterial cell as the product of a grand series of molecular self-associations? The notion is so seductive, and so deeply embedded in molecular philosophy (see [6] for a recent example), that it must be explicitly refuted again and again [1,2,3]. Microbiologists were compelled to abandon molecular self-assembly thirty years ago, when it became clear that peptidoglycan cell walls are not composed of discrete subunits. The sacculus, at least that of *E. coli*, consists of a single giant macromolecule whose enlargement requires cutting and splicing, a procedure more akin to weaving than to assembly [7,8,9]. Cells do, of course, assemble themselves, but in another sense of the word: they grow. Growth does not flow down the thermodynamic hill; on the contrary, growth proceeds steadily away from equilibrium, thanks to the continuous input of both energy and information. Growth depends on a network of chemical processes, many of which display both location and direction in cell space, that collectively convert energy into organization.

**Cell walls and surface stress**

The paramount role of the cell wall in bacterial morphogenesis became apparent some forty years ago. Wall fragments retain the general shape of the cell from which they came; conversely, enzymatic digestion of the wall (in the presence of osmotic support) produces spherical protoplasts or spheroplasts. Bacterial cells are turgid, and it is the wall that resists the pressure, keeps the cell from bursting and comes under stress. The chief stress-bearing element is the peptidoglycan sacculus, a cross-linked network of strands whose properties resemble those of a stiff strong fabric, not unlike nylon mesh. The bacterial peptidoglycans are all very much alike (with some differences in detail), and supply no chemical basis for the cells’ very existence proved to be a pointer to places where gene-specified traits intersect with generic biophysics. Application to particular organisms requires additional postulates, and the surface-stress theory was purposely formulated around the minimal number of indispensable assumptions. It explains how the work is done, and constitutes a framework for thinking about bacterial forms in general. Application to particular organisms requires additional postulates, and has achieved varying degrees of success. It is noteworthy that a significant number of experimental findings were either anticipated by the theory, or found a logical place within it. The list includes alternating synthesis of wall and septum in rods, dispersed growth of lateral walls, inert poles and inside-out growth. And in a few favorable cases it has actually been possible to calculate the shape that cells should have, including the poles of *Bacillus subtilis* and the entire outline of dividing *Enterococcus hirae*. That still seems to me a remarkable achievement, with few parallels in the annals of morphology.

On the debit side, the surface-stress theory has little to say about the many mutants whose shape is aberrant. Their very existence proved to be a pointer to places where gene-specified traits intersect with generic biophysics. We now know that bacteria possess a cytoskeleton after all, which functions both in septum formation and in the localization of lateral wall synthesis. Cell division is almost certainly guided, not by soap-bubble physics but by a molecular machine (see below). All the same, I suspect that these new discoveries complement the role of wall biophysics, without superseding the parts played by turgor pressure and by wall expansion. A more realistic theory of morphogenesis will still incorporate several principles that first entered our subject by way of Arthur Koch’s calculator.
**Arranged by a cytoskeleton**

In just the past decade, our image of bacterial cells has been radically transformed: where once we saw chiefly the random jostling of free—wheeling molecules, we now recognize structured systems, where critical molecules own an address and many functions require the participants to be in the right place at the right time. In prokaryotes as in eukaryotes, a dynamic cytoskeleton pervades and integrates cell space and confers harmony on the hubbub of molecules. Thanks to the spate of recent discoveries, we now know most of the players on the stage of morphogenesis, and have a much better sense of how the plot unfolds. Briefly, the shape of the cells is maintained by their wall, the peptidoglycan layer in particular, which supplies an exoskeleton. The wall is shaped by the pattern of peptidoglycan synthesis, localized in space and in time. The enzymes of wall biosynthesis, and many other components as well, are positioned by the cytoskeleton. The coherence of cellular operations is a gift of that cytoskeleton.

The transformation began in the ’nineties, with the discovery that the protein FtsZ, a component of the machinery of cell division, assembles into a ring at the site of cytokinesis [16]. It is now clear that construction of the septum is effected by a multi-molecular complex commonly called the divisome. The ring of proteins constricts, closing the partition between the daughter cells; and as it constricts, it draws unto itself the deposition of new wall and plasma membrane that will eventually make up two new cell poles. We do not yet understand how the divisome directs wall biosynthesis, what makes it constrict, and why pole wall is metabolically so inert. On the face of it, the divisome looks like a molecular device that performs mechanical work in opposition to the expansive force of turgor pressure. A stream of reviews tracks the primary literature, many of them quite excellent [17,18,19,20,21].

The mechanism by which rod-shaped cells locate their mid-point, and block unwanted septum formation elsewhere, has become the focus of intense and competitive research (reviewed in [20,21,22,23,24,25]). In *E. coli*, this is achieved by proteins of the Min family, which oscillate between cell poles and inhibit septum construction. The time-averaged concentration of the inhibitors is highest at the poles and lowest midway between them, which is why the septum is initiated there. Oscillation is not brought about by diffusion, but by alternating expansion and collapse of a dynamic scaffold. Curiously, this mechanism is by no means universal: *B. subtilis*, for example, relies on Min proteins but not on oscillation, while *Caulobacter crescentus* apparently lacks Min proteins altogether.

The lateral walls of rod-shaped cells are produced by a distinct set of synthases, directed by a separate cytoskeletal apparatus [26]. MreB and its homologs are related to eukaryotic actin rather than tubulin, and assemble into dynamic helical filaments along the cell’s axis. Good reviews summarize the torrent of new findings [21,24,27]. There is strong evidence that these filaments localize peptidoglycan synthases, conferring a helical topology upon wall synthesis (rather than dispersed, as thought previously). The actin-like cytoskeleton plays a role in various aspects of cell shape, and perhaps in chromosome segregation too. For example, in *Caulobacter* it is required for the expression of cell polarity and works together with the protein crescentin to generate the cells’ distinctive curved morphology (reviewed in [21,24,27]). What is still quite uncertain (at least to me) is just how these filaments function: could they serve as tracks for the transport of macromolecules, or even of vesicles, as actin filaments do in eukaryotes?

There is surely much more to come, and it is likely to reinforce the perception that, as in eukaryotes, the cytoskeleton constitutes the primary agent of cytoplasmic order. As a spectator, frequently bewildered, I hope that future work will clarify the relationship of the various filamentous structures to each other, and present a more comprehensible picture of the cytoskeleton as a whole. I hope to learn what is so special about pole peptidoglycan that makes it inert [15,28], and how bacteria manage to keep a constant girth, and just what advantage they derive from their shapes (for a remarkable effort along that line see [29]). And I look forward to making sense of the surprising fact that bacteria operate with a diversity of cytoskeletons. But now we must hurry on to peer around the next corner: who or what arranges the cytoskeleton?

**It takes a cell to shape a cell**

We do not presently know enough about the cytoskeleton to specify in any detail how its organization comes about, but I will offer an opinion. I think that we will find that the bacterial cytoskeleton, like its eukaryotic counterpart, is produced by a variety of self-organized assembly processes, guided by instructions supplied by the cell as a whole, by way of heritable membranes, spatial markers and other features of cell continuity [3,10].

The more we learn about bacterial morphogenesis, the clearer it becomes that growing cells construct themselves upon the existing structural framework. Much of our current knowledge comes from the research program initiated years ago by Lucy Shapiro (and continued by herself, former students and collaborators) on the life cycle of *C. crescentus*, which is visibly polarized (reviewed, among others, in [24,29,30,31,32,33]). An important general insight is that cell poles serve as organizing centers: a flock of signal-transducing proteins are dynamically
localized to one pole or the other at specific stages of the cell cycle. The actin-like cytoskeleton plays a prominent role in the polarized localization of proteins, possibly supplying transport tracks that communicate with markers deposited at each pole. Two such markers, TipN and TipF, have recently been identified [34,35]. They are clearly instrumental in defining a pole, since in their absence poles do not form properly, and when mislocalized they cause poles to form at the wrong site. Now, TipN and TipF are proteins, specified by their cognate genes; but to function correctly they must also be in the right place. As I read the findings, pole proteins display cell polarity, and also help to maintain and execute it; but no single protein is the cause of polarity. Polarized growth is perpetuated through each division by the deposition of the appropriate markers, which keep the cycle going. Asymmetry is a feature of the system as a whole, and present continuously; and the crucial step that regenerates asymmetry at each division is the construction of two fresh poles.

When asked to explain our objectives, students of morphogenesis sometimes reply that we seek to understand how genetic information is translated into temporal and spatial organization. Obviously, much remains to be learned, but it seems to me that the crucial discovery was made long ago: the translation can only take place in the context of a whole cell. Spatial organization is not spelled out by particular genes; it arises epigenetically by the interaction of numerous gene products, within the structural framework of a living cell. Every cell is continuous with its progenitor, not only genetically but also architecturally. Offspring resemble their progenitors because they share the same genes, but also because they are made upon the same last. There’s a whiff of vitalism about this attitude, a note of deep heresy, but it should not occasion surprise. A century and a half ago, Rudolf Virchow proclaimed that every cell comes from a previous cell, and nothing has happened since to suggest that he was mistaken. What we are now discovering, in bacteria as well as in eukaryotes, is just how cells go about making cells.

Conclusions

Are we there yet? How will we know when, at least in principle, we understand how bacterial shapes are generated? With regard to simpler puzzles, such as protein synthesis or oxidative phosphorylation, that point was reached long ago. Open questions remain and new discoveries continue to be made, but one can explain to a class of bright undergraduates how the trick is done. By that criterion, our understanding of bacterial morphogenesis remains deficient, but it is visibly superior to what it was a decade ago. The next steps, however, may prove painful: we will, I believe, be compelled to lay aside the illusions of reductionism and put the cell back in the center.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


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A pioneering effort to understand why bacteria have the shapes by which we know them.


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