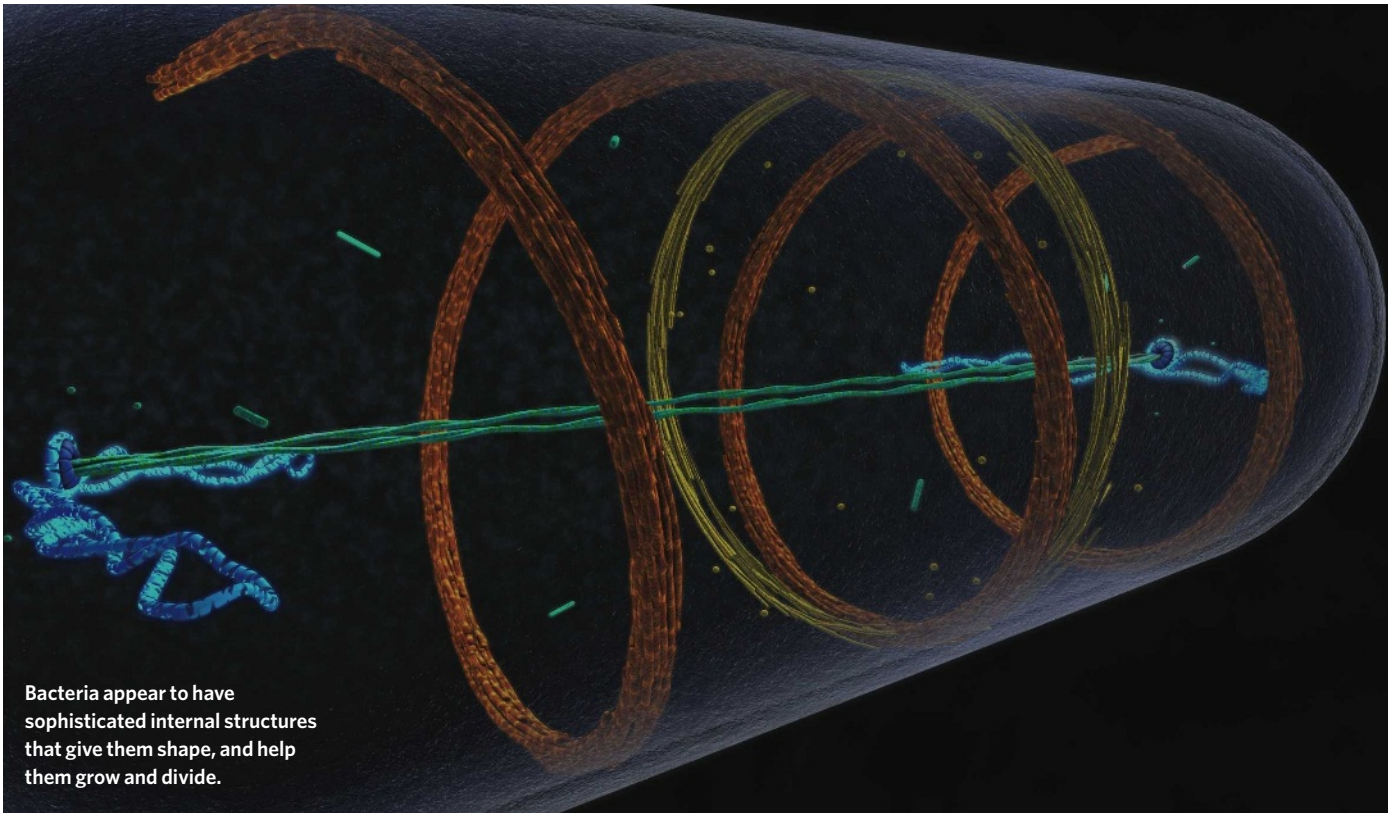
BACTERIA'S NEW BONES

Long dismissed as featureless, disorganized sacks, bacteria are now revealing a multitude of elegant internal structures. **Ewen Callaway** investigates a new field in cell biology.

Nearly a decade ago Jeff Errington, a microbiologist at Newcastle University in England, was toying with a strange bacterial protein known as MreB. Take it away from microbes, and they lose their characteristic cylindrical shape. The protein's obvious role in structure and even its sequence suggested a shared ancestry with actin, a protein that produces vast, fibrous networks in complex cells, forming the framework of their internal structure, or cytoskeleton. But no one had ever seen MreB in action under the microscope until Errington found just the right combination of fluorescent labels and fixatives.

In a 2001 paper, he presented MreB (orange in the illustration below) fluorescing brilliantly and painting barbershop-pole stripes around the rod-shaped bacterium *Bacillus subtilis*[1]. "We got these amazing pictures. It was one of those few times in a scientific career when you do an experiment that completely changes your way of thinking," says Errington.

For more than a century, cell biology had been practised on 'proper' cells — those of the eukaryotes (a category that includes animals, plants, protists and fungi). The defining characteristic of eukaryotic cells is their galaxy of internal structures: from the pore-studded nucleus that contains the genome, to the fatty sacs of the Golgi, to the myriad mitochondria, and of course the networks of protein highways that ferry things around the cell and give it shape and the capacity for movement. These elements form a catalogue of cell biology's greatest discoveries, and all of them are absent in bacteria. Hundreds to thousands of times smaller than their eukaryotic cousins, and seemingly featureless, bacteria were rarely invited to the cell biology party. But Errington's discovery has been part of a movement that is changing that.

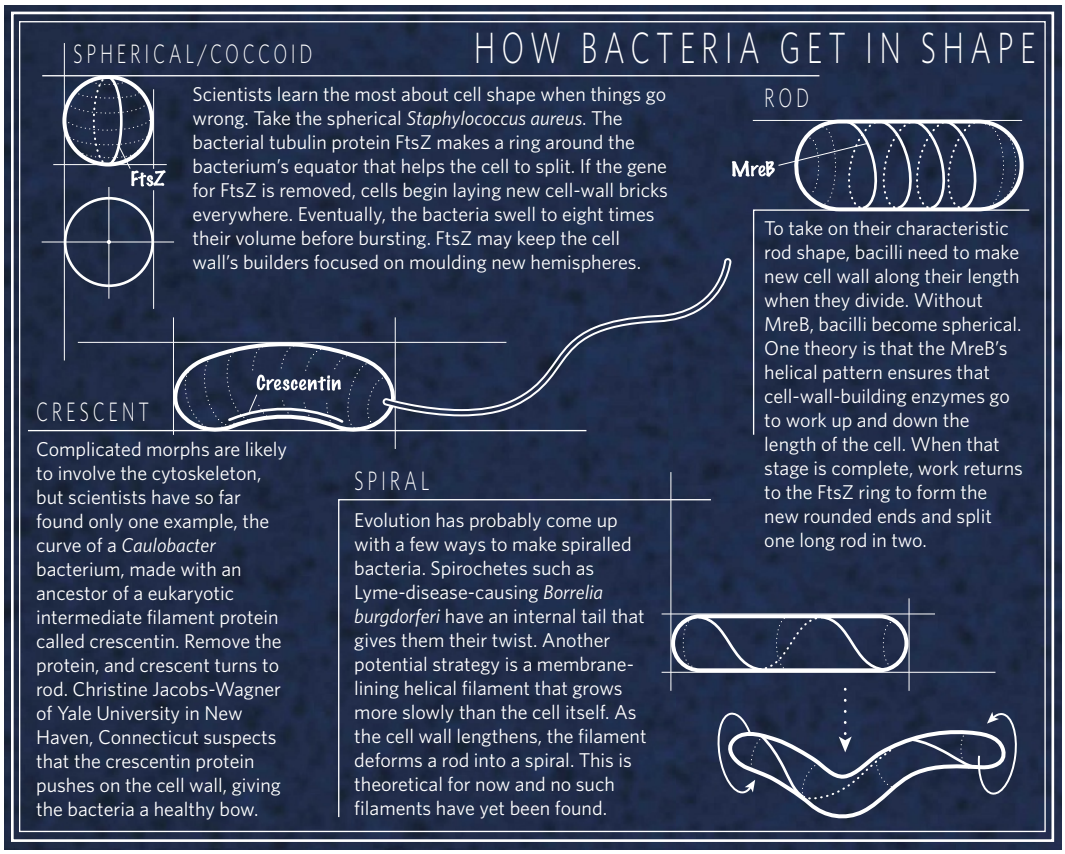


J. IWASA

Bacteria appear to have sophisticated internal structures that give them shape, and help them grow and divide.

Dyche Mullins, a cell biologist at the University of California, San Francisco had spent most of his career untangling the network of molecular cables and scaffolding that enforces order in the eukaryotic cell. With Errington's paper, Mullins saw the lowly bacterium anew. "There was a lot of organization in bacterial cells we were just missing," he says. He has since devoted much of his time to studying them. Last month, Mullins chaired the annual meeting of the American Society for Cell Biology in Washington DC. That he was chosen for the job is a clear indication that bacteria have made it on to the guest list.

Lucy Shapiro, a microbiologist at Stanford University in California gave bacteria an hour-long tribute at the meeting. "People more or less thought the bacterial cell was a swimming pool and the chromosome was this ball of spaghetti," says Shapiro, whom many credit for launching the field of bacterial cell biology.



External contractors

Busied by growth, propagation and little else, a bacterium's life can seem an endless cycle of fecundity. Well-fed bacteria in rich, sterile culture can divide every half hour or so. The cytoskeleton is the linchpin of efficient cell growth and division, but researchers are only starting to explain how.

Take FtsZ (illustrated in yellow opposite), a protein that ties a belt around the belly of nearly every species of bacterium. Without FtsZ — a barely recognizable cousin of the eukaryotic protein tubulin — rod-shaped bacteria called bacilli grow longer and longer without splitting in two. Somehow FtsZ cinches the dividing cell closed, says Harold Erickson, a cell biologist at Duke University Medical Center in Durham, North Carolina. Tubulin is involved in eukaryote cell division, but its role is completely different. Microtubules, formed from tubulin, pull chromosomes apart during cell division through a process that has been studied extensively.

Erickson started out studying tubulin. But intrigued by the pictures of internal FtsZ structures coming out of other labs in the 1990s, he began reading up on FtsZ. When the time came to reapply for a grant, he devoted half of his proposal to the bacterial protein. "I decided, 'I don't have any great ideas about what to do with tubulin,'" he recalls. It took a couple of applications to get funding, but Erickson hasn't looked back.

Squeezing two cells out of one is just one of the cytoskeleton's duties. When bacteria divide they need to resculpt a rigid cell wall built out of peptidoglycan, a polymer consisting of sugar and amino-acid bricks. Without the MreB protein wound around the shell of a bacillus, it grows spherical (see 'How bacteria get in shape'). The protein directs the construction and destruction of the cell wall, says Zemer Gitai, a microbiologist at Princeton University in New Jersey. One theory is that MreB and its relatives build a protein scaffold inside the cytoplasm that tells the cell wall's enzyme contractors outside the cytoplasm where to lay new bricks. Because two layers of membrane separate the MreB helix from the cell wall, other proteins must forge the connection, says Gitai.

Also, when a bacterium divides, each new cell must have its own DNA. Most of a bacterium's thousand or so genes sit on a long chromosome, but smaller rings of DNA called plasmids also help a cell by supplying antibiotic resistance and other perks.

Mullins's lab studies a bacterial version of actin, called ParM, which ensures that as a cell splits in two, each receives a copy of a specific plasmid. Without the protein, many cells will invariably lose the plasmid and the drug resistance it provides.

To avoid this fate, a strand of ParM molecules (shown in green, opposite) latches onto two freshly replicated plasmids (purple), like the chain to a pair of handcuffs. The two circles start close to one another, but as more ParM molecules leap onto the chain, the plasmids spread to opposite ends of the cell. Mullins's group found that the ParM chain grows pretty much on its own — a startling contrast to our own actin, which requires other players to speed extension. Although related to actin, ParM works more like tubulin, constantly reinventing itself by adding and shedding units. "That blows my mind," Mullins says.

His team is now looking at how other plasmids ensure their legacy, to say nothing of the bacterial chromosome, a DNA loop thousands of times longer than any individual plasmid. "We know very little. For me, the most important unanswered question in cell biology is how bacteria segregate their chromosomes," says Mullins.

The wealth of questions and dearth of answers makes the field very attractive. Every time a new bacterium is sequenced, research-

ers have the opportunity to find new structural elements, often with surprising roles. One of the latest additions is an actin protein, MamK, found in bacteria endowed with iron-containing structures called magnetosomes. By sensing Earth's magnetic tug, the bacteria can position themselves in the environment best suited to their needs. For the compass to work, a cell's dozen or so magnetosomes need to line up in a row, and MamK forms their track[2]. Arash Komeili, a microbiologist at the University of California, Berkeley who first identified the protein's role says that by scouring genome databases he has found genes similar to MamK in bacteria with no magnetosomes.

Seeing is believing

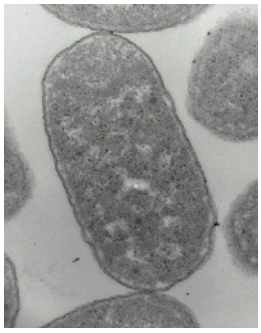
Although bacterial cell biologists such as Komeili can use genomics to hunt for new features of the cytoskeleton, pictures make a stronger case, he says. Advances in optics and microscopy are one reason the bacterial cell is only now getting its dues. At a few micrometres, bacteria are often not much longer than the limits of a light microscope, so even the best lens in the world won't bring any detail to a molecular cable a few nanometres thick.

Peering deeper into a bacterial cell requires abandoning the light waves that obscure detail. Electrons, which have a far shorter wavelength than visible light, provide staggering insights into eukaryotic cell structure, such as the ribosome-studded endoplasmic reticulum or the perfectly arranged bundle of microtubules that build a cilium tail. In bacteria, the same electrons paint a blurry mush. Even the most recent edition of the hallowed text *Molecular Biology of the Cell* sees bacteria under the magnification of an electron microscope as chaotic vessels: "This cell interior appears as a matrix of varying texture without any obvious organized internal structure," the authors write.

A more promising technology — cryo-electron tomography — might be the answer. Instead of coating cells with gold or dousing them in harsh fixatives, cryo-EM, as it is often called, takes pictures of flash-frozen samples. "We're looking at cells in a nearly native state," says Grant Jensen, a biologist at California Institute of Technology in Pasadena. The gentle treatment keeps the bacterial cytoskeleton intact. "If you thawed them out, most of them would probably swim away."

Cryo-EM has the added benefit of allowing researchers to combine numerous angles of a cell into a three-dimensional picture, just like a computed tomography scan does. Recently, Jensen's lab collected images of rings of FtsZ lining the insides of a bacterium called *Caulobacter* and pinching its membrane — a model predicted by others but never seen before.

K. LOUNATMAA/SPL



Electron microscopy suggests that *Echerichia coli* and other bacteria have no organized internal structure.

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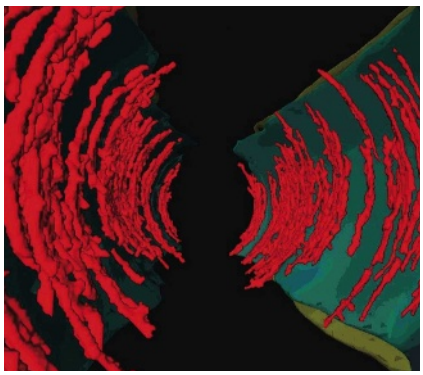

Cryo-electron tomography of a mutant *Caulobacter* shows gobs of FtsZ filaments (red) lining the constriction site as the cell tries to divide.

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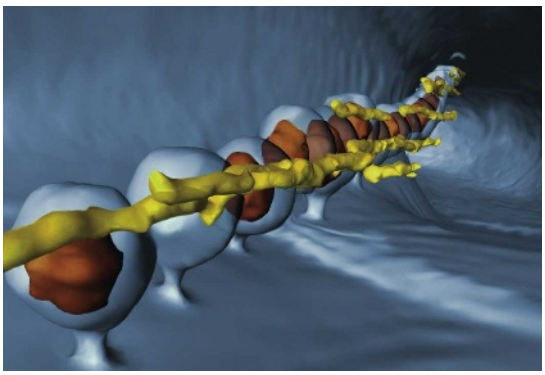

An actin-like filament called MamK (yellow) organizes a chain of magnetosomes (iron-containing structures) in the magnetic bacterium *Magnetospirillum magnetotacticum*.

When early searches for bacterial genes resembling eukaryote scaffold-protein genes found nothing, scientists assumed that these proteins evolved after bacteria split from eukaryotes, some 1.5 billion to 2 billion years ago. The discovery of the bacterial cytoskeleton has turned that conclusion on its head.

FtsZ may be the great-grandfather of cell division, says Erickson, whose lab recently showed that the protein makes rings inside microscopic droplets of oil, a stand-in for early life. Although cell division now is an elaborate choreography between dozens of players, the earliest cells may have needed just FtsZ to split in two. Erickson points out that the protein contains none of the amino acids, such as tryptophan and arginine, that some believe only to have shown up later in evolution.

As cytoskeletons evolved, they took on new chores and snowballed in complexity. At some stage after eukaryotes branched off from bacteria, the eukaryote cytoskeleton seems to have frozen in time. From yeast through to people, its proteins do many of the same jobs, such as towing sister chromosomes to opposite ends of a dividing cell or making sure the endoplasmic reticulum nestles up against the nucleus. More complex eukaryotes might use actin to flex muscles and keratin to make hair, but those tasks are variations on a theme.

Not so with bacteria, says Mullins. Actins that determine cell shape work differently across the bacterial world, and some rod-shaped bacteria, such as tuberculosis, don't even have them. Due to their vast numbers and unicellular lifestyle, "bacteria can play around with fundamental mechanisms for doing things in a way that eukaryotes can't", he says.

But the shared trait of bacterial and eukaryotic cytoskeleton proteins — self assembly — means that bacteria can shed light on the workings of more complex species. For example, the molecular structure of MreB explained how actin molecules stick together. And in most cases, bacterial proteins yield to laboratory tinkering with less resistance than the eukaryotic kind. Turning up the expression of actin, for instance, kills many eukaryotic cells, but bacteria don't seem to mind.

And bacteria, because they have few genes, are ideal for addressing fundamental questions about all cellular life. Although cytoskeletons seem to act as organizing centres in bacteria and eukaryotes, no one yet understands how these proteins travel to precise spots in a cell, to one end or the other or to the site where one cell splits in two.

As well as being intellectually stimulating, probing the insides of bacteria has practical applications, and bacterial cell biologists recognize the need to remind funding agencies such as the National Institutes of Health of that. For example, a chemical named A22 slows bacterial growth by stopping MreB from forming into long cables, and without FtsZ many bacteria will die. No antibiotics yet target the bacterial cytoskeleton, but with drug resistance on the rise, structures such as the MreB helix and the FtsZ ring could prove to be chinks in the bacterial armour.

But as researchers struggle to piece together the bacterial cell, cures for disease are far from the minds of most. For Mullins, the field's progress has vindicated his dive into the bacterial swimming pool, although he and others still haven't come close to its deep end. "There's a lot of unexplored biology," he says. ■

Ewen Callaway recently completed an internship at *Nature*'s Washington DC office.

1. Jones, L. J. F., Carballido-López, R. & Errington, J. *Cell* **104,** 913–922 (2001).
2. Komeili, A., Li, Z., Newman, D. K. & Jensen, G. J. *Science* **311,** 242–245 (2006).