tion of the carbon isotope ratios in 3.8- to 3.3-billion-year-old sediments from Greenland, South Africa, and Australia as signs of photosynthetic microorganisms (*10*–*15*) has also been called into question (*4*).

Organic carbon molecules produced by microorganisms leave typical degradation products in rocks, and it was on the basis of such molecules that the oldest molecular evidence for oxygenic photosynthesis was identified in 2.7-billion-year-old oil-rich rocks in the Pilbara (*16*), now reidentified as recent contamination (*17*). In rocks almost a billion years older, the molecules are even more degraded, and there is little to distinguish them from the prebiotic organic molecules found in meteorites. Nevertheless, measurements on 3.5-million-year-old samples from the Pilbara have shown that small-scale structural details of the conformation of certain organic molecules (such as a predominance of odd over even carbon numbers in spectra produced by pyrolysis gas chromatograph-mass spectrometry of the remnant carbon) can be traced back to living organisms (*18*). However, these measurements were performed on a bulk rock sample, and there is no context information about the environment of formation of the rocks or the kinds of life forms they could contain.

In contrast to the macroscopically identifiable stromatolites, other microbial signatures are far more subtle and hence more challenging to identify in the rock record, although they are more common. Biolaminated sediments, for instance, are formed by the rhythmic alternation of sticky photosynthetic microbial mats, formed on the surfaces of inter- to supratidal sediments, and intervening layers of sediment (*19*, *20*) (see the figure). The resulting textural signatures include laminations caused by the stabilization of sediment surfaces, rippled and ripped-up mats produced by wave action, or even desiccation cracks in exposed mats. Compositionally, the layers may have higher carbon contents. These structural, textural, and compositional signatures of the nonstromatolite-forming microbial mats can be preserved in the rock record.

Silicified biolaminated sediments have been identified in 3.5- to 2.9-billion-year-old rocks in South Africa and Australia (*11*, *12*, *15*, *20*, *21*). From 3.4-billion-year-old sediments in the Barberton greenstone belt in South Africa, Tice and Lowe (*12*) recorded portions of microbial mats, formed in shallow littoral waters, that have been broken up by physical stress and redeposited in deeper water environments as rolled-up fragments (*11*). In the same area, we have documented overturning and mechanical shearing of a 3.3-billion-year-old filamentous microbial mat under flowing water (*15*). Filaments in the latter mat had average diameters of 0.25 μm, with lengths reaching several tens of micrometers. Portions of resedimented mats from the Pilbara contained similar-sized filaments in 3.4-billion-year-old intertidal sediments (*14*). Such filaments, and other microorganisms (*14*), may be characteristic of life at that period: that is, anaerobic and small in size.

Searching for signatures of life in the oldest well-preserved sediments is difficult because of degradation of the materials and the pitfalls represented by confusing abiogenic biosignature mimics. And because life was small and anaerobic, its signatures are subtle and more challenging to identify unambiguously. However, the past half decade has seen a breakthrough in the methods used to identify biosignatures, opening the way for a future that will reveal the profusion of life on an anaerobic planet.

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CELL BIOLOGY

Protein Filaments Caught in the Act

Grant J. Jensen

Advances in electron microscopy have allowed bacterial DNA-segregating protein filaments to be visualized.

Cells can be thought of as little chemical processing plants, but they also accomplish some marvelous physical and mechanical tasks such as shaping themselves into characteristic forms, moving toward nutrients, organizing their complex interiors, replicating and then segregating their DNA, and dividing (*1*). It has long been understood how in eukaryotes most of this work is done by cytoskeletal filaments—long protein polymers that are used like cables, tracks, and beams in the machinery of the cell. But until about a decade ago, it was a mystery as to how bacterial cells did the same tasks. None of the existing technologies, including "traditional" electron microscopy methods, had convincingly revealed analogous cytoskeletal filaments in bacteria. As a result, the lack of a cytoskeleton became widely regarded as a distinguishing characteristic of prokaryotic cells. Now, on page 509 of this issue (*2*), Salje *et al*. show direct images of an important bacterial cytoskeletal filament responsible for DNA segregation.

The findings of Salje *et al*. add to a series of discoveries that have firmly debunked the idea that prokaryotes lack a cytoskeleton (*3*). First, improvements in light and immunoelectron microscopy led to the identification of several bacterial proteins whose elongated localization patterns suggested that they were polymerizing into filaments (*4*). Next, a series of stunning crystal structures showed that many of these proteins had the same structures as known eukaryotic cytoskeletal proteins (*5*). In vitro biochemistry then demonstrated how some of these proteins did in fact form dynamic filaments with all the properties required to perform cytoskeletal functions (*6*). But seeing is believing, and the development of cryoelectron microscopy (cryo-EM) methods has in just the past few years allowed a number of bacterial cytoskeletal filaments to be imaged directly, inside cells, doing their jobs.

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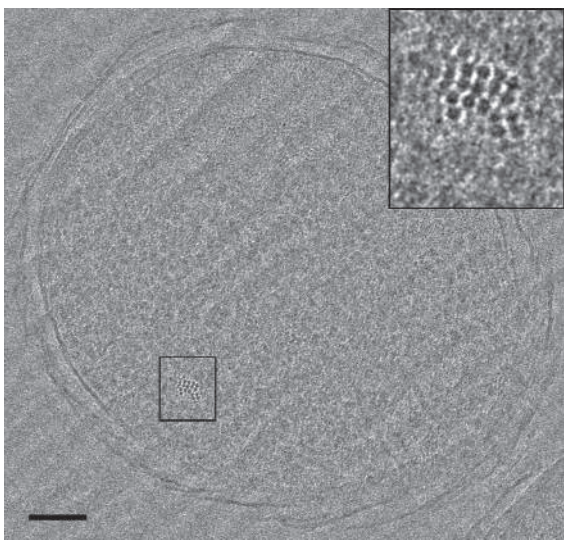
The key was that the development of cryo-EM methods allowed samples to be imaged frozen in a near-native, lifelike state, thus bypassing the harsh preparative procedures of chemical fixation, dehydration, plastic embedment, and staining required by traditional electron microscopy. Because cytoskeletal filaments have now been seen frequently within bacteria through cryo-EM techniques, it appears that the harsher "traditional" techniques simply failed to preserve such fine structures. As a second major recent advance, electron tomographic methods have been developed that allow entire small cells, not just sections of cells, to be imaged in three dimensions (*7*). This allows filaments that bend and curve, and therefore might be missed in a single planar section, to be recognized and followed.



Freeze frame. Cryo-EM projection image of a vitreous section of an *E. coli* cell containing a high-copy plasmid with the ParMRC segregation system. A single bundle of ~16 ParM cytoskeletal filaments is seen at the lower left (and **inset**), cut in cross section and projected down the filaments' long axis so they appear as small dots. Scale bar, 100 nm. [Reproduced from (*2*)]

Unfortunately, although these advances have opened a completely new window into the ultrastructure of several bacterial species (*8*), they were not immediately applicable to the bacterium *Escherichia coli* because the high-energy electrons typically used in cryo-EM can only penetrate about 0.5 μm of biological material before being inelastically scattered (and thus lost to the image). *E. coli* cells are, unfortunately, just larger than this, and are therefore problematically thick. This is a major disappointment, because *E. coli* is by far the most studied bacterium (and possibly the most studied cell of any type), and images of its putative cytoskeleton are in high demand, as so much is already known about its complex cell biology. To overcome this challenge, Salje *et al*. first cryosectioned frozen *E. coli* cells and then imaged the frozen sections. This resulted in the first direct in vivo images of an *E. coli* cytoskeletal filament, the plasmid-segregating protein ParM.

ParM is part of the simplest cellular DNA (plasmid) segregation system discovered to date, involving only two proteins: ParM, which self-assembles into a dynamic filament, and ParR, an adaptor protein that anchors the tips of ParM filaments to plasmids at a special short DNA sequence called parC. ParM filaments segregate plasmids by binding through ParR to two identical copies of the plasmid (one at each end of the filament), growing until they extend across the cell from one pole to the other, and then releasing the plasmids near the poles. This greatly improves the chances that when the cell then divides at its midplane, each daughter cell will receive its own copy of the plasmid (*9*). Although these points had all been established previously, ParM filament bundles had never actually been seen directly inside cells. Salje *et al*. froze cells at high pressure (which prevents formation of large ice crystals that would have distorted the cellular ultrastructure), cryosectioned them to produce slabs thin enough for cryo-EM, and then recorded either projection images or full tomograms of the frozen sections—a strategy that has been named CEMOVIS ("cryo-EM of vitreous sections") (*10*). Filament bundles were unambiguously recognized in the images.

Although cryo-EM methods do allow cellular structures to be imaged in a native state, there are as yet no effective labels that can be used to identify molecules of interest. Previous studies had identified specific protein filaments by varying the expression level of a candidate protein (i.e., from absent to highly overexpressed) or the stability of the filament it formed, and then observing which filaments in the cell exhibited corresponding changes in their number or length (*11*, *12*). Salje *et al*. did the same, imaging cells overexpressing ParM protein alone, cells harboring high-copy-number and then low-copy-number plasmids bearing the ParMRC machinery, and finally control cells lacking ParM entirely. In a technological first, however, Salje *et al*. further strengthened their case by showing that the putative ParM filaments they saw had the same characteristic spacings between filaments, and between monomers along a single filament, as did ParM filaments assembled in vitro from purified ParM protein. In the cells harboring the low-copy-number plasmid, they occasionally saw small bundles of just three to five filaments near the edge of the nucleoid (the nucleus-like region in the cytoplasm of a prokaryotic cell where DNA localizes). Biologically, these images strongly support the model that there is one filament for each plasmid pair (*13*), and further reveal that the filaments and plasmids are somehow bundled together at the edge of the nucleoid (see the figure).

These findings point the way toward new questions and opportunities. It is unclear, for instance, how the filaments are bundled together, or why the ParM filaments were consistently seen within the periphery of the nucleoid. Similar cryosectioning approaches may allow images of the proteins FtsZ, MreB, MinCDE, and other putative cytoskeletal machinery in *E. coli* to be obtained (although each will present its own special challenges because of their different abundances, positions, curvature, and sizes). Analyses of the characteristic spacings (structural "signatures") of other filaments may help identify them, just as it did ParM. Finally, as one of a burst of pioneering applications of CEMOVIS, the study of Salje *et al.* further justifies hopes that we will one day be able to produce three-dimensional maps of even large (eukaryotic) cells to this same degree of "molecular resolution" through tomography of serial vitreous sections.

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