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 photo-
synthetic 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 evi-
dence for oxygenic photosynthesis was identi-
fied in 2.7-billion-year-old oil-rich rocks in the 
Pilbara (16), now reidentified as recent con-
tamination (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 meteor-
ites. Nevertheless, measurements on 3.5-mil-
lion-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 car-
bon 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 envi-
ronment of formation of the rocks or the kinds 
of life forms they could contain. 

In contrast to the macroscopically identifi-
able 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 alterna-
tion 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. Com-
positionally, the layers may have higher carbon 
contents. These structural, textural, and compo-
sitional 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 environ-
ments 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 con-
tained similar-sized filaments in 3.4-billion-
year-old intertidal sediments (14). Such fila-
ments, and other microorganisms (14), may be 
characteristic of life at that period: that is, 
aerobic 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 abio-
genic biosignature mimics. And because life 
was small and anaerobic, its signatures are 
subtle and more challenging to identify un-
ambiguously. 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.

## REFERENCES

2. N. McLoughlin et al., Geobiology 6, 95 (2008).

## 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 ac-
complish some marvelous physical and mechanical tasks such as shaping them-
several 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 cyto-
skeletal 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 fil-
aments with all the properties required to per-
corm 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.
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 embedding, 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 to bend and curve, and therefore might be missed in a single planar section, to be recognized and followed.

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 filament structures 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 bundles 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.

References