

JENSEN LAB

CELL ATLAS

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Introduction

In the 1960s, electron microscopes were opening a new window in biology, allowing scientists to look not just at cells, but into them. This revealed a rich world of ultrastructures too fine to resolve with light microscopes, including organelles inside eukaryotic cells. To share this new vista with scientists and medical students who didn't have microscopes to look for themselves, authors like Don Fawcett and John Dodge created atlases of electron microscopy images that remain valuable resources for biology and medical novices, as well as experts.

Fifty years later, we are once again enjoying an expanded view of biology, thanks to another great advance in electron microscopy. The development of cryogenic electron microscopy, or cryoEM, allows us to look inside cells in their native state, without the sample dehydration, staining and embedding required previously. This has opened up even the smallest cells for examination, and revealed some surprising things. Bacteria and archaea in particular, orders of magnitude smaller than eukaryotic cells and lacking prominent organelles, previously seemed to be relatively unstructured bags of nucleic acids and protein. In the last decade, cryoEM has challenged this view, revealing a startling degree of structure in these tiny cells. And so, inspired by the atlases of eukaryotic cell structure from the 1960s, we offer an atlas of bacterial and archaeal cell structure.

Just as the technology of electron microscopy has advanced in the intervening decades, the technology of sharing information has similarly evolved. Taking advantage of a digital medium, we can share not just two-dimensional slices through cells, but their full three-dimensional volumes. This medium also allows you to tailor your experience. If you want a basic overview, simply follow the main narrative. If you want to go into more depth on a topic, follow the “More” links to see additional examples and details. If you’re interested in comparative evolution, explore the book from the phylogenetic tree. If you’re interested in a particular structure, explore from the index.

If you’re new to cryoEM, we suggest starting with Chapter 1, which describes the methods used in structural biology, particularly cryoEM. If you’re already an expert, or pressed for time, go straight to the

cells in Chapter 2. Before you do, though, please watch this short introductory video.

[1_1_Introduction]

As Charles Darwin wrote in 1837, “I shall always feel respect for every one who has written a book, let it be what it may, for I had no idea of the trouble which trying to write common English could cost one.” The task was made immeasurably easier for us by the help of many minds and hands Acknowledgments.

Acknowledgments

We are grateful to Rob Phillips, our colleague at Caltech, for introducing Grant to Fawcett’s Atlas and encouraging him to create another. Readers of early drafts gave us useful feedback to improve this experience. In particular, we thank Lydia Jensen and Natalie Jensen, who acted as test readers, as well as many past and present Jensen Lab members for advice and feedback. We are grateful to Ashley Jensen and Tony Kukavica for help with research. We are deeply grateful to Travis Alvarez, Camille Ogilvie, Natalie Jensen and Aditee Prabhutendolkar, who created most of the movies. And we are most grateful of all to our colleagues whose work at the microscope filled these pages. Click on their names throughout the book to learn a little bit more about them.

We used the IMOD software package (developed by David Mastronarde, Rick Gaudette, Sue Held, Jim Kremer, Quanren Xiong, John Heumann and others at the University of Colorado with support from the NIH) to create and visualize tomographic datasets, and we are grateful to David Mastronarde for tireless support, including improving a function to help us make these movies. We used UCSF Chimera (developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41 GM103311) to create the visualizations of atomic models from the Worldwide Protein Data Bank (wwPDB). We thank Lam Nguyen for generating the atomic model of a lipid bilayer. Schematics and atomic models of proteins are the work of many labs; full references appear at the end of each chapter.

The Caltech Library, particularly Thomas Morrell, Kristin Briney, Stephen Davison, and Donna Wrublewski, supported and enabled our vision of open access publishing and we are enormously grateful for their work and ingenuity in creating a platform tailored to the content and our shared vision of open accessibility. The textbook is built on the excellent bookdown platform from Rstudio (Xie (2015)).

1

Methods

1.1 *Light microscopy*

1.2 *Fluorescence light microscopy*

1.3 *Electron microscopy*

1.4 *Thin-section TEM*

1.5 *Cryo-EM*

1.6 *Cryo-ET*

1.7 *Subtomogram averaging*

1.8 *CLEM*

1.9 *Single-particle reconstruction*

1.10 *X-ray crystallography*

1.11 *Visual proteomics*

2

Envelope

2.1 *Mycoplasma genitalium*

The fundamental unit of life is the cell – a contained self-replicating assembly. For many species, including all *Bacteria* and *Archaea*, the organism consists of a single cell. And for nearly all species, no matter how many cells an organism eventually contains (probably around 10 trillion in your case), it started life as a single cell. As you'll see, the details of these cells vary, but every cell on Earth is the same at heart – a DNA-based replicator machine built from just four macromolecules: nucleic acids, proteins, lipids and carbohydrates.

Imagine that you're a structural engineer tasked with building one of these cells. What's the first step? Let's start with the container. No matter what the first self-replicating molecules were (likely RNA), they didn't constitute a cell until they became packaged in a container. You'd probably want a flexible container that allowed you to sort a subset of molecules from the environment. Evolution agrees. All cells are enclosed by a selectively permeable membrane, made of lipids and proteins Schematic – Lipid bilayer, that allows them to differentiate their contents from the environment. This selectivity is a critical feature for the life of the cell Schematic – ATP synthase. The compartment enclosed by a cell's membrane is called the cytoplasm ("cell mold" [the membrane being the container that shapes the mold]).

Almost all archaea and many bacteria, like these *Mycoplasma genitalium* cells, are monoderms ("single skin"). This means that their cytoplasm is enclosed by a single membrane. At the resolution of this image, the membrane looks like a single dark line, but remember that it's really a bilayer, as you'll see in some later images.

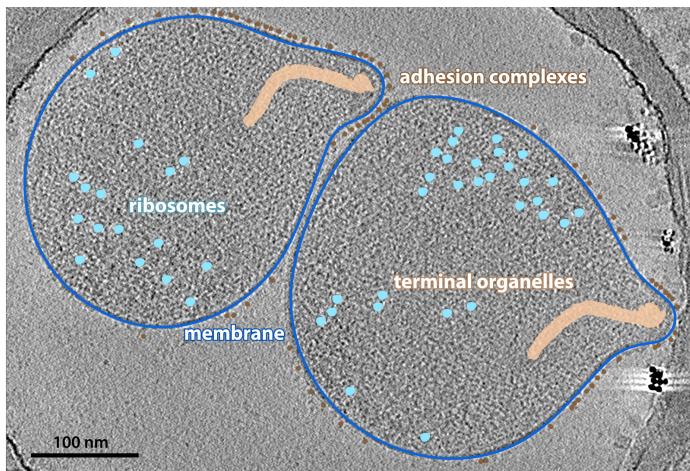
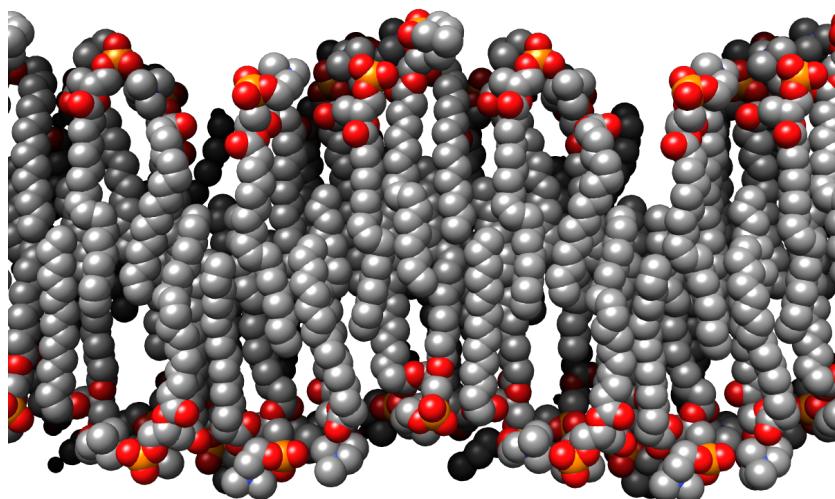


Figure 2.1: Mycoplasma genitalium
Collected by: Gregory Henderson
[10.22002/D1.1350](<https://doi.org/10.22002/D1.1350>)

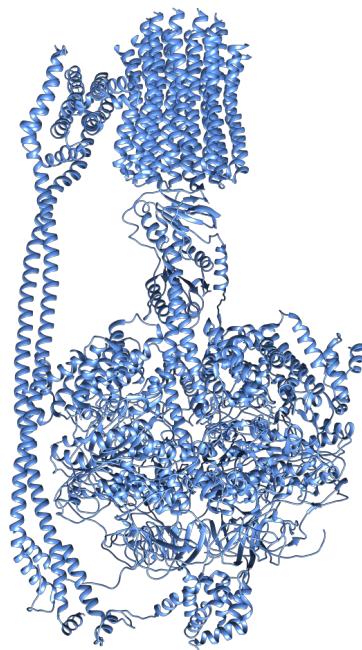
2.1.1 Lipid bilayer



Lipids have a hydrophilic head (red/orange in the schematic) and hydrophobic tails (gray); in water they spontaneously pack together side-by-side to shield their tails from unfavorable interactions with water, forming closed double-layered bags. Proteins with regions of amino acids containing hydrophobic side groups embed these regions in lipid bilayers. Other proteins are fused to lipids, tethering them to

the membrane. In fact, cells' "lipid" membranes actually constitute roughly equal fractions of lipids and proteins. One key difference between archaea and bacteria (and with them, eukaryotes) is the lipid that makes up their membranes. Hybrid membranes containing both these lipid types can be made artificially, and it's possible that the last universal common ancestor of all cells on Earth contained both types, with specialization later occurring in different lineages.

2.1.2 ATP synthase and energy production



The chemical properties of lipids make membranes impermeable to ions and large or hydrophilic molecules (but not to water). Cells take advantage of this property to establish an ion gradient across the membrane, using a chain of electron-carrying proteins in the membrane to pump protons out of the cell. Protein complexes in the membrane called ATP synthases (like this one from *Escherichia coli*) use the resulting ion potential to generate energy. The machine provides a conduit for protons to flow down their potential, producing a "proton-motive force" that spins the machine's rotor, generating energy that is chemically stored in ATP, the energetic currency of the cell.

2.2 *Listeria monocytogenes*

Being able to selectively move things into your cell enables it to do some powerful things. It also poses a structural problem, though. Remember that water can pass freely through the membrane, which means that increasing the solute concentration inside relative to the environment will cause water to rush in as well, introducing a pressure (known as turgor pressure) on the membrane. Lipid bilayers, though, are unable to withstand much pressure. If your cell lives exclusively in a consistent, and fairly high-osmolarity, environment (like our bodies, in the case of the *Mycoplasma genitalium* you just saw), it can balance internal and external osmolarity to minimize turgor pressure on the membrane. But most cells experience much more variable environments. How can you keep your cell from bursting in such conditions?

You'll probably want to add some rigid scaffolding outside the membrane to buttress it against turgor pressure. Nearly all bacteria do this using a material called peptidoglycan: long stiff polymers of glycan sugars crosslinked by short peptides into a chain-mail-like mesh. The full scaffold of this material surrounding the cell is called its sacculus, or cell wall. Some archaea also have cell walls, made of a molecule similar to peptidoglycan, but chemically distinct. Most archaea, though, rely on a different structure for support, as you'll see later in this chapter.

In monoderm bacteria like this *Listeria monocytogenes*, the cell wall is significantly thicker than the membrane. It comprises several layers of peptidoglycan, which can't be seen individually at the resolution of this image, so the cell wall appears as a uniformly textured layer More: Peptidoglycan architecture. It's still a mystery how large molecules can pass through this dense layer on their way to and from the cell. Not all bacterial cell walls are chemically identical More: *Methanobacterium formicum*. And in some conditions, cells can lose their walls Schematic – L-form bacteria.

2.2.1 *Bacillus subtilis*

The sacculus is so robust that it persists even after cells are lysed and their other components digested. This sacculus isolated from a *Bacillus subtilis* cell has retained its shape, simply flattening with the release of contents and pressure from inside. By observing how these purified sacci rip and curl, we can infer something about the architecture of the cell wall; we think that the long glycan strands are oriented in hoops circling the short axis of the cylindrical cell and the short peptide crosslinks are more or less aligned with the long axis.

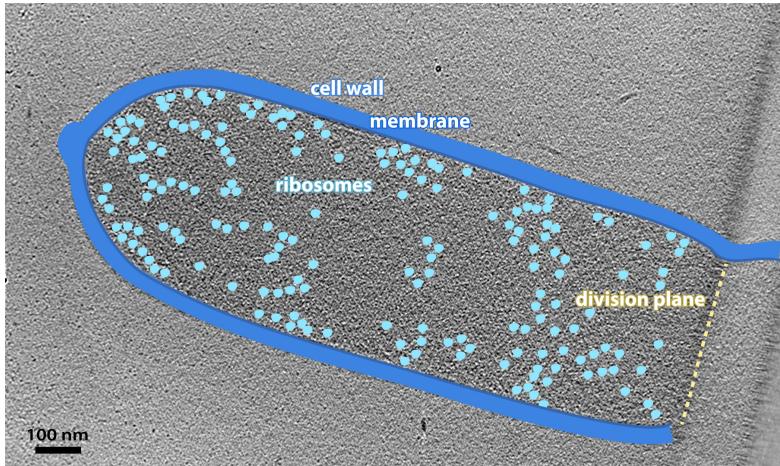


Figure 2.2: *Listeria monocytogenes* Collected by: Ariane Briegel [10.22002/D1.1351] (<https://doi.org/10.22002/D1.1351>)

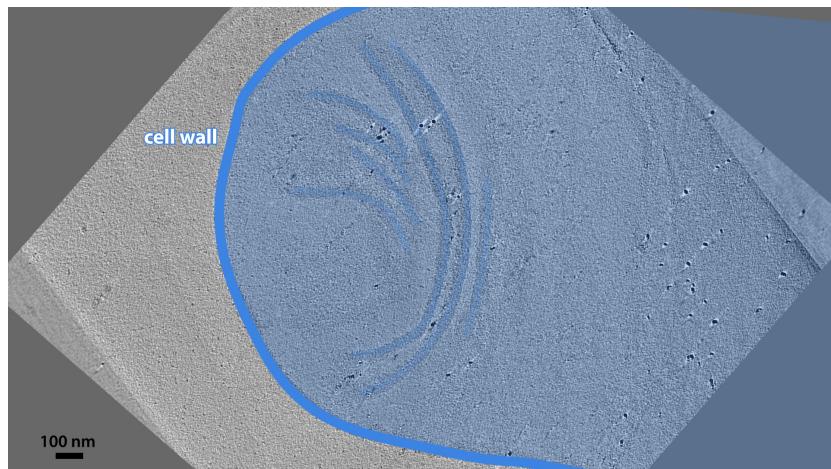
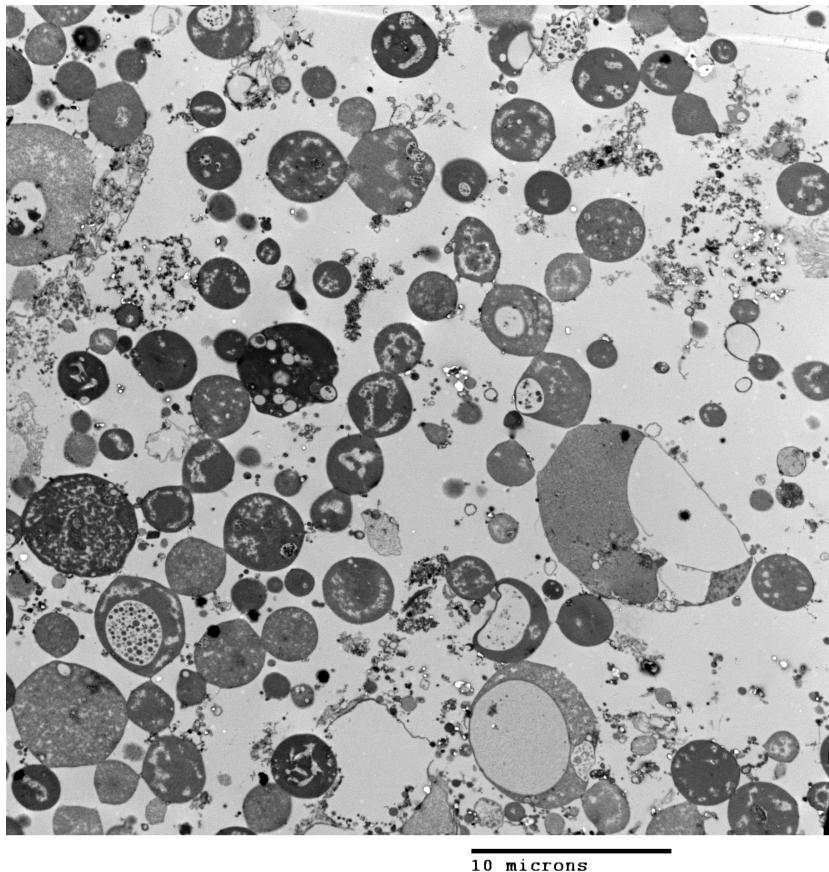


Figure 2.3: *Bacillus subtilis* Collected by: Morgan Beeby [10.22002/D1.1360] (<https://doi.org/10.22002/D1.1360>)

2.2.2 *L-form bacteria*

There is no universal adaptation in Nature; advantages in one environment can become liabilities in another. This concept is exemplified by an adaptation of some bacteria which lose their cell walls in certain conditions, such as in the presence of antibiotics (the cell wall is a common target of antibiotics). This state is called the L-form (named for the Lister Institute where it were discovered). As you can see in these *Bacillus subtilis*, cells in this state are pleomorphic, exhibiting a variety of sizes and shapes. As you would expect, L-form cells are more sensitive to environmental conditions. In the lab, they're protected from lysis by increasing the osmotic pressure of the environment, for instance by adding sucrose. The environment in your body, though, would have the same effect, as we discussed for *Mycoplasma genitalium*. L-form bacteria are interesting for many reasons (including human health), one of which is that they give us a fascinating window into how early cells – prior to the evolution of the cell wall – might have looked and behaved.

2.3 *Cupriavidus necator*

Why stop at one membrane, though? Think about how the bacteria you've just seen compare with eukaryotic cells. The eukaryotic cells are much larger (maybe 100-1,000 times larger in volume), and they contain many internal membranes that form specialized subcompartments, like the nucleus and mitochondria. Bacteria and archaea don't have membrane-bound organelles inside, but in fact many bacteria do create an additional compartment outside the cell with another, outer, membrane. These bacteria, like the *Cupriavidus necator* cell you see here, are called diderms ("double skin"). The extra compartment between their membranes is known as the periplasm ("mold between"). This antechamber contains a unique subset of proteins, many of which function in escorting things into and out of the main cell, as you'll see in later chapters.

Compared to the inner membrane, the outer membrane has some unique properties! It is more permeable and not proton-tight (so it can't be used to generate ATP). It is often asymmetric, with a different composition of lipids and proteins in each of the two leaflets. The outer membrane is anchored to the sacculus Schematic – Braun's lipoprotein. And the sacculus itself is different. Rather than containing many layers of peptidoglycan like in the *Listeria monocytogenes* cell you just saw, the diderm sacculus consists largely of a single-layered peptidoglycan mesh More: Diderm sacculus architecture, which you can see here as a thin line in the periplasm. This difference in the sacculus enables a well-known bacterial classification system: the Gram stain, which binds peptidoglycan. Gram-positive cells, typically monoderm, contain much more peptidoglycan than Gram-negative cells, which are typically diderm. Thinking about this thin defense against turgor pressure underscores a major challenge for cell growth. To insert new material, existing bonds in the sacculus must be broken, without bursting the cell in the process Schematic – Sacculus remodeling.

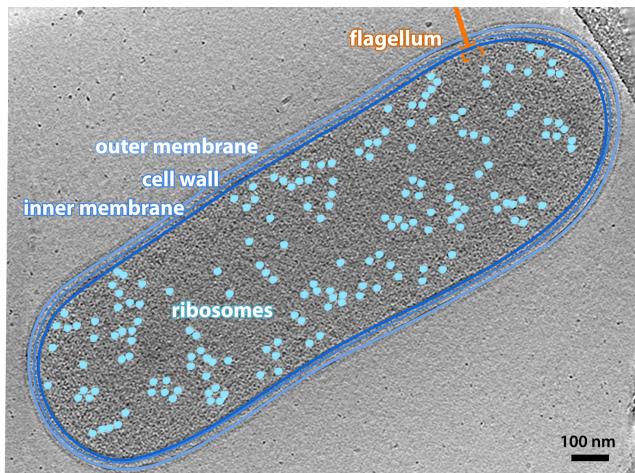
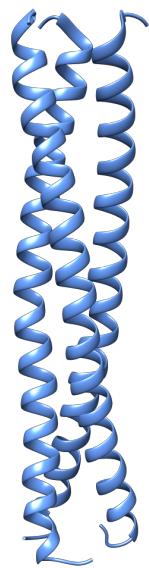


Figure 2.4: *Hydrogenovibrio crunogenus*
Collected by: Cristina Iancu
[10.22002/D1.1352](<https://doi.org/10.22002/D1.1352>)

2.3.1 Braun's lipoprotein



Lipoproteins are hybrid molecules, formed by covalently linked lipid and protein pieces. The lipid allows them to embed into a membrane, tethering the attached protein to function nearby. Braun's lipoprotein, which is one of the most abundant molecules in the outer membrane of cells like *Escherichia coli*, uses its tethered protein portion to bind the peptidoglycan cell wall, creating a link between the outer membrane

and the cell wall (adding up, for a typical *E. coli* cell, to about 100,000 links). These links determine the distance between these two components. Not all diderms use Braun's lipoprotein, though, and some bacteria have notably labile outer membranes More: Outer membrane lability.

2.3.2 *Escherichia coli*

Compare this diderm sacculus purified from *Escherichia coli* to the monoderm sacculus on the last page. Since this one is thinner; we can make out more details. Instead of inferring how the glycan strands are oriented, we can now see them running around the circumference of the cell. The main difference between the two types of sacci seems to be whether they have largely a single layer of peptidoglycan (diderm) or many layers (monoderm). So even though the two cell walls look different, their architecture is fundamentally the same. In some circumstances, cells can even switch between the two forms, as you'll see in Chapter 7.

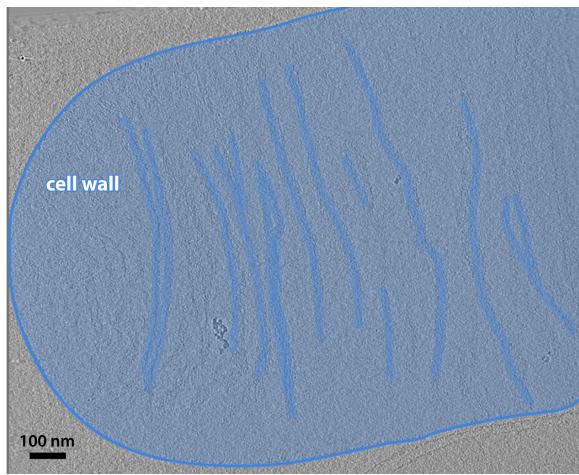
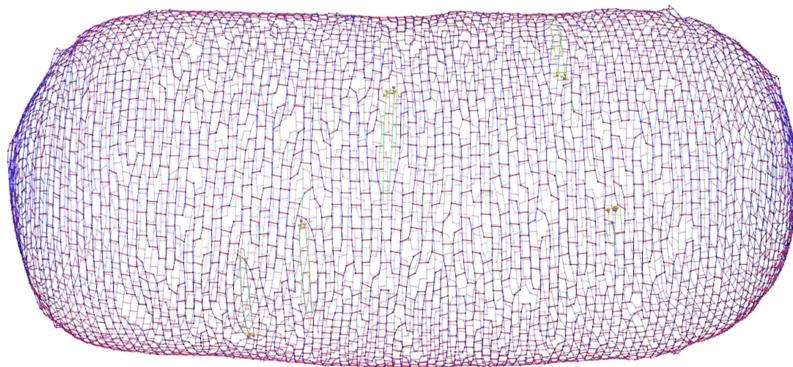


Figure 2.5: *Escherichia coli* Collected by: Lu Gan [10.22002/D1.1362](<https://doi.org/10.22002/D1.1362>)

2.3.3 *Sacculus remodeling*



Encasing your cell in a rigid scaffold presents a problem: how can it grow? It's easy to make membranes larger simply by adding more lipids. But to add more peptidoglycan strands, they have to be linked into the existing network, which means breaking existing links to accommodate them. In fact, cells remodel their sacculi with the tools you'd expect: an enzyme that links glycan sugars into strands, an enzyme that links strands together with peptide bonds, and an enzyme that cuts these peptide links. Remember, though, that your cell, with its solute-rich interior, has a turgor pressure pushing outward with a force of maybe 3 atmospheres, equivalent to what we would feel at a depth of 20 meters in the ocean. This is more than enough to lyse an exposed bacterial membrane. So these tools must be used carefully to ensure that the cell doesn't burst. We're still figuring out exactly how this works, with help from computer simulations like this one. Here you see a model of an *Escherichia coli* sacculus being enlarged using the three enzyme tools we just described. This simulation was run to test whether just having the tools function in a complex rather than separately might give enough coordination for smooth, safe growth. (The answer was yes.)

More: Diderm archaea Nearly all diderm cells are bacteria. Not all, though. As you see here, *Ignicoccus hospitalis*, an archaeon, has an outer membrane that appears very loosely associated with the cell, forming an extra large periplasm. You can see membrane-bound vesicles shuttling cargo across this vast space (more about vesicles on the next page). Interestingly, this is also an exception to the rule that the inner membrane of diderms is “energized” (proton-tight). In *I. hospitalis*, it is the outer membrane that contains the ATP synthases. These unusual characteristics suggest a symbiotic origin for this species; perhaps its ancestor used to live inside a host cell, which was eventually reduced to a mere membrane.

2.4 *Borrelia burgdorferi*

What else can you do with an extra membrane? Since membranes make excellent containers for molecules, why not get into the shipping business? In the coming chapters (especially Chapter 8), you'll see some of the ways that cells interact with each other and their environment. For diderm bacteria, many of these interactions are made possible by outer membrane vesicles ("little bladders")—self-contained pockets budded off the membrane. The vesicles may carry cargo of antibiotics to inhibit competitors' growth, or toxins to lyse neighboring cells. Or enzymes to digest those lysed remains into nutrients that your cell can easily take up as food. Alternatively, they may carry emergency kits (first aid and survival factors) for other members of a community biofilm. The appearance of these vesicles varies as much as their contents. More: Vesicle morphologies. They are usually spherical, of a consistent size, and often come off the cell at one or a few sites, forming chains, as you can see in this *Borrelia burgdorferi* cell.

Not all diderms produce outer membrane vesicles, and even for the ones that do, we still don't know exactly how they do it. Maybe it happens spontaneously due to the physics of lipids and proteins in a certain configuration. Or maybe there's a dedicated protein machine in the membrane, blowing bubbles. Some archaea (monoderms) also produce membrane vesicles. They're less studied than their bacterial counterparts, but likely serve similar roles in metabolism and community interactions.

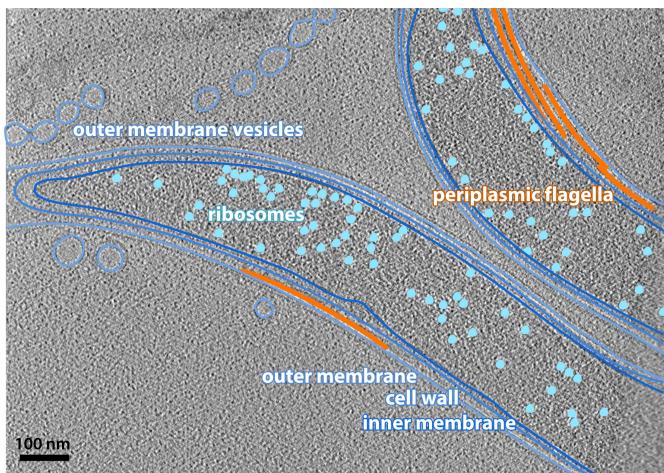


Figure 2.6: *Borrelia burgdorferi* Collected by: Ariane Briegel [10.22002/D1.1353](<https://doi.org/10.22002/D1.1353>)

2.4.1 *Borrelia burgdorferi*

Different species can produce outer membrane vesicles that look very different. The same species can also produce vesicles that look very different. Sometimes they come off the cell as a chain of spheres; sometimes the spheres remain connected, like a string of pearls; sometimes vesicles form long tubes instead, like from this *Borrelia burgdorferi* cell. Sometimes the same chain can be tubular in one section (usually at the base, connected to the cell), and a string of spheres in another.



Figure 2.7: *Borrelia burgdorferi* 2 Collected by: Ariane Briegel [10.22002/D1.1363] (<https://doi.org/10.22002/D1.1363>)

2.4.2 *Myxococcus xanthus*

Not all vesicles come from the outer membrane. The cytoplasmic or inner membrane can also form vesicles that are released into the cytoplasm, as in this *Myxococcus xanthus* cell, or into the periplasm. This seems to be a less regulated process than outer membrane vesicle formation, and we see it in many species when they are stressed by low nutrients or high cell density, suggesting that it is a general phenomenon. Cells shrink in harsh conditions (more on that in Chapter 7), so cytoplasmic or periplasmic vesicles may simply offer a place to put the extra membrane or, more optimistically, to store it until the time comes to grow again. Just as with outer membrane vesicles, the appearance of cytoplasmic vesicles can vary widely More: Cytoplasmic vesicle variety.

2.4.3 *Prosthecobacter debontii*

Cytoplasmic vesicles exhibit a variety of sizes and shapes. Some are nested, with vesicles inside vesicles. In this *Prosthecobacter debontii*

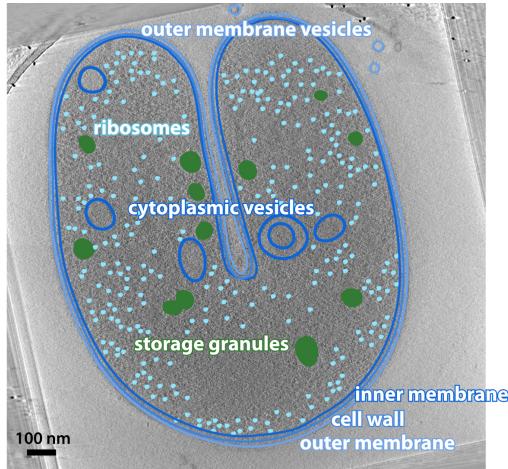


Figure 2.8: *Myxococcus xanthus*
Collected by: Matthew Swulius
[10.22002/D1.1364](<https://doi.org/10.22002/D1.1364>)

cell, you can see two other morphologies. One is a large, flattened horseshoe-shaped vesicle. Another is a more typical spherical shape, but is decorated with what looks like protein complexes.

This cell also has unusual structures on its surface that have yet to be identified.

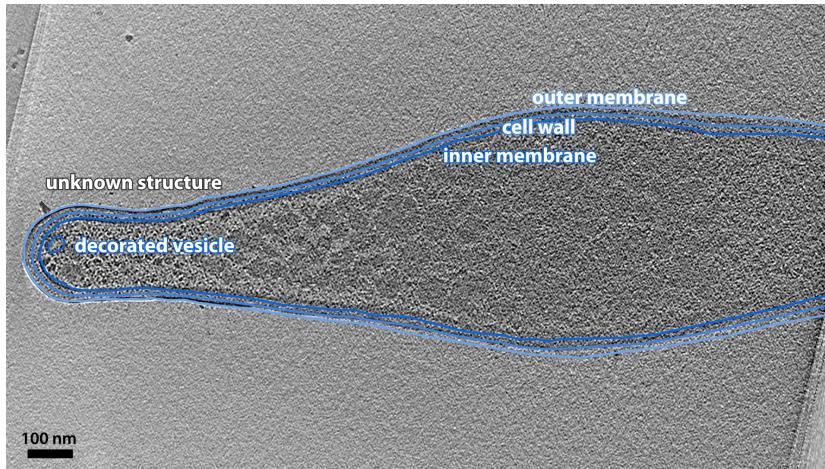


Figure 2.9: *Prosthecobacter debontii* Collected by: Martin Pilhofer
[10.22002/D1.1365](<https://doi.org/10.22002/D1.1365>)

2.5 *Mycoplasma marinum*

Evolution is endlessly creative, providing exceptions to nearly every classification rule. We've just described a neat breakdown of bacteria into monoderms (one membrane, thick saccus, positive Gram stain) and diderms (two membranes, thin saccus, negative Gram stain). But some cells, like this *Mycoplasma marinum*, defy classification.

Mycobacteria are diderm, with an inner and an outer membrane, and a cell wall. But they have unique molecules (named mycolic acids in their honor) in the outer membrane. These acids interfere with Gram staining, yielding an intermediate result between positive and negative. And their sacculus consists of three layers, each with a unique molecular composition Schematic – Mycobacterial architecture. In this case, the middle layer is the familiar peptidoglycan.

When you look at the schematic, you'll see another layer outside the outer membrane. This layer, the capsule, isn't visible in the movie of the cell, though. It is made up of "Extracellular Polymeric Substance," abbreviated EPS, or long chains of sugars, sometimes linked to the outer membrane and sometimes free-floating. These sugars don't interact strongly with electrons, so the capsule is usually invisible by electron microscopy. But in fact, many bacteria (mostly diderm, but also some monoderm) have a capsule. The capsule can help bacteria attach onto surfaces and offers an extra layer of protection, trapping water to prevent desiccation and making it more difficult for hydrophobic molecules like detergents to get through to disrupt the membranes. It also makes it more difficult for viruses to reach the cell, and for eukaryotic "predators" like macrophages to eat it.

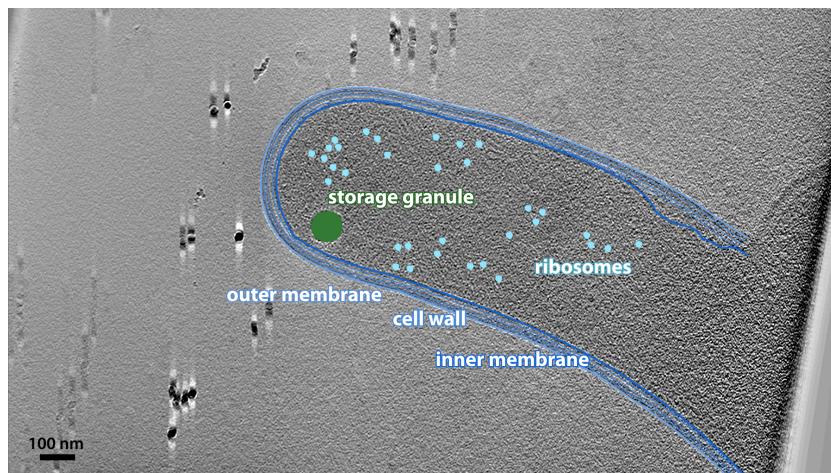
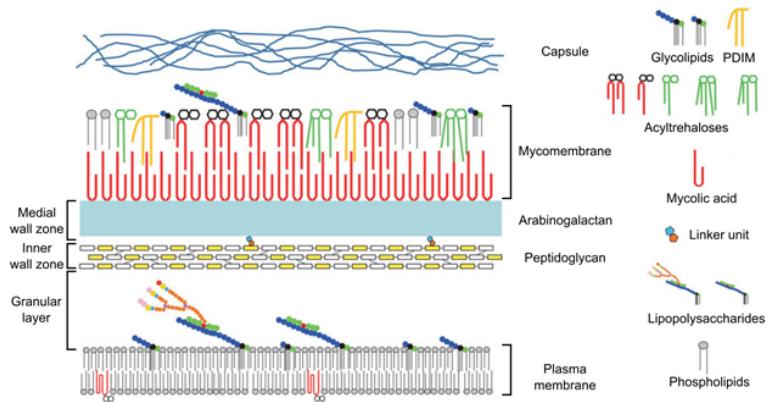


Figure 2.10: *Mycobacterium marinum* Collected by: Elitza Tocheva [10.22002/D1.1354] (<https://doi.org/10.22002/D1.1354>)

2.5.1 Mycobacterial architecture



A unique architecture encloses Mycobacterial cells. Note the outer membrane, with lipids only in the outer leaflet, and mycolic acids forming the inner. Note also the multiple strata of sugars in the cell wall. Also note the capsule depicted outside the outer membrane.

2.6 *Caulobacter crescentus*

How else can you protect your cell from the rigors of a harsh world? What about encasing it in an armored shell a la the armadillo? Many bacteria (both monoderms and diderms) and archaea use modular proteins for this purpose, interlocking Lego-block-like pieces into a shell called the Surface Layer, or S-layer. You can guess that this must offer a significant evolutionary advantage since up to 15% of the total protein in the cell can be found in the structure. In fact, S-layers play many roles for cells, some of which you'll see on the next page, but one of their main functions is as a gatekeeper, preventing large things like viruses from reaching the membrane.

S-layers are crystalline lattices, and they can be striking in appearance, as on this *Caulobacter crescentus* cell. Amazingly, the lattice is made from (many copies of) a single protein Schematic – S-layer architecture. The pinwheel-like subunits interact laterally, leaving pores large enough for nutrients to pass through, but not large enough for viruses. The modular nature of the lattice means that units can be popped in as the cell grows, or popped out to allow a cell appendage to poke through. In general, S-layers are quite accommodating; they don't even interfere with the production of outer membrane vesicles More: Nanopods. S-layer proteins can also be modified to alter their properties; for instance attachment of a sugar can enable them to stick the cell to a surface.

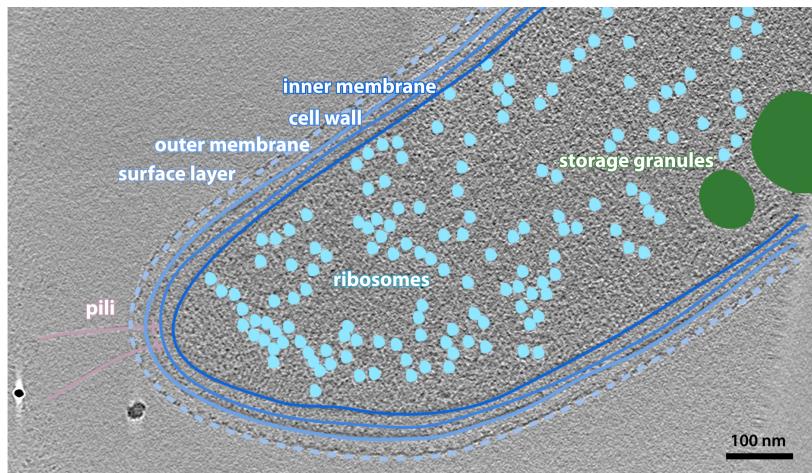
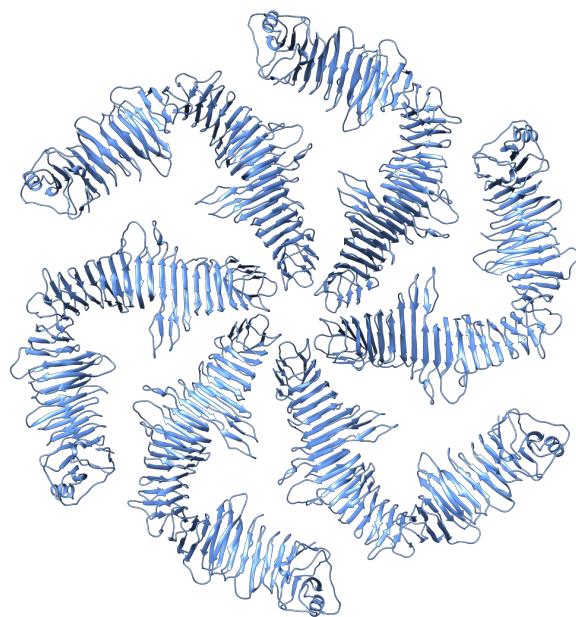


Figure 2.11: *Caulobacter crescentus*
Collected by: Ariane Briegel
[10.22002/D1.1355](<https://doi.org/10.22002/D1.1355>)

2.6.1 S-layer architecture



A single protein forms the S-layer you just saw in *Caulobacter crescentus*. The protein has two domains. The bottom domain anchors to lipoproteins attached to the outer membrane. The top domain forms the canopy of the S-layer, organizing hierarchically into hexameric rosettes like this that in turn pack into a larger hexameric lattice. This lattice is flexible enough to curve around even narrow regions of

the cell (more on this stalk in Chapter 3).

2.6.2 *Delftia acidovorans*

Archaea and diderm bacteria with S-layers produce characteristic outer membrane vesicles: they bud off with the S-layer attached. *Delftia acidovorans*, like this produce so-called nanopods: chains of outer membrane vesicles ensheathed in S-layer.

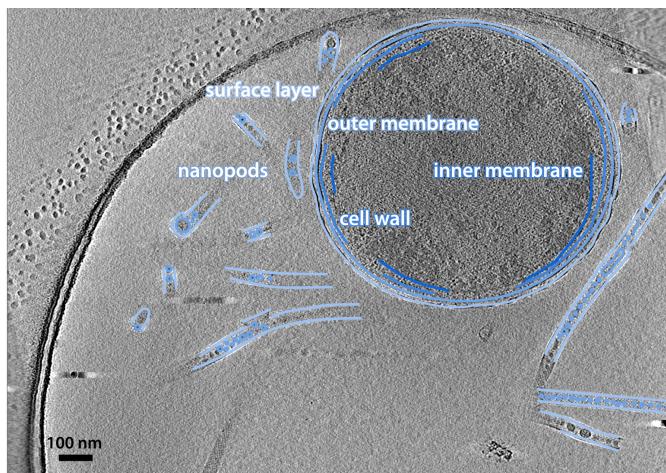


Figure 2.12: *Delftia acidovorans*
Collected by: Elitza Tocheva
[10.22002/D1.1366](<https://doi.org/10.22002/D1.1366>)

2.7 *Sulfolobus solfataricus*

One of the most striking features of the S-layer is how different it can look in different species. For instance, compare this archaeal *Sulfolobus solfataricus* cell to the bacterium on the last page, or to other diderm More: *M. alcaliphilum* or monoderm More: *C. thermocellum* bacteria, or archaea More: *N. maritimus* More: *Methanoregula formicina*. All S-layers are crystalline lattices of a single—or in a few cases, two—proteins, but the particular pattern of the lattice depends on the shape of this building block and how it multimerizes into a higher-order structure. The shape of the building block varies considerably; there is almost no sequence homology between S-layer proteins from different species. And shapes come together in different ways, forming repeating units of one, two, three (as on this cell), four, or six blocks.

S-layers are very common in archaea like this cell. Most archaea don't have cell walls, but the S-layer provides the same function of external scaffolding. Remember, too, that nearly all archaea are monoderms, lacking the extra periplasmic compartment that diderms have. Here again the S-layer serves a similar function, enclosing a space around the cell's membrane called the pseudo-periplasmic space. Just

as with the bacterial periplasm, this space serves as an antechamber for the cell, restricting access by large molecules. In some cases, the pseudo-periplasmic space also contains specific proteins that function in metabolism.

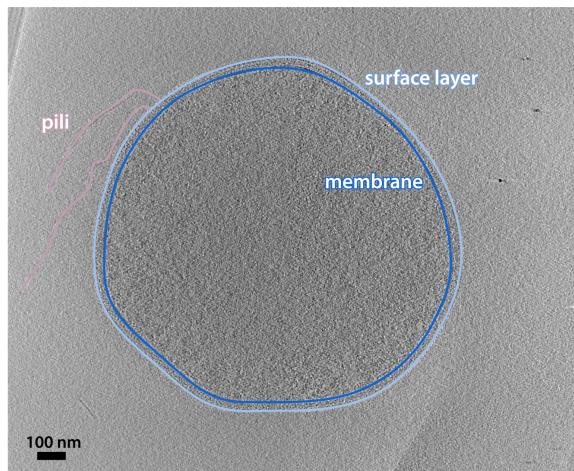


Figure 2.13: *Sulfolobus solfataricus* Collected by: Lu Gan [10.22002/D1.1356](<https://doi.org/10.22002/D1.1356>)

2.7.1 *Methylomicrobium alcaliphilum*

In *Methylomicrobium alcaliphilum*, V-shaped S-layer proteins come together to form cups that pack into a hexagonal pattern.

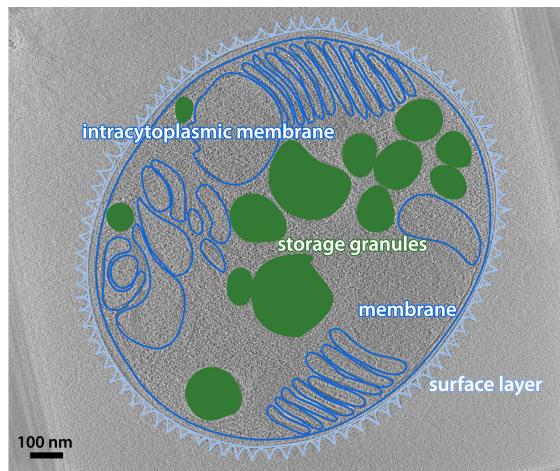


Figure 2.14: *Methylomicrobium alcaliphilum* Collected by: Songye Chen [10.22002/D1.1367](<https://doi.org/10.22002/D1.1367>)

2.7.2 *Nitrosopumilis maritimus*

In *Nitrosopumilis maritimus*, S-layer proteins form hexagonal rosettes that in turn pack into a hexagonal lattice.

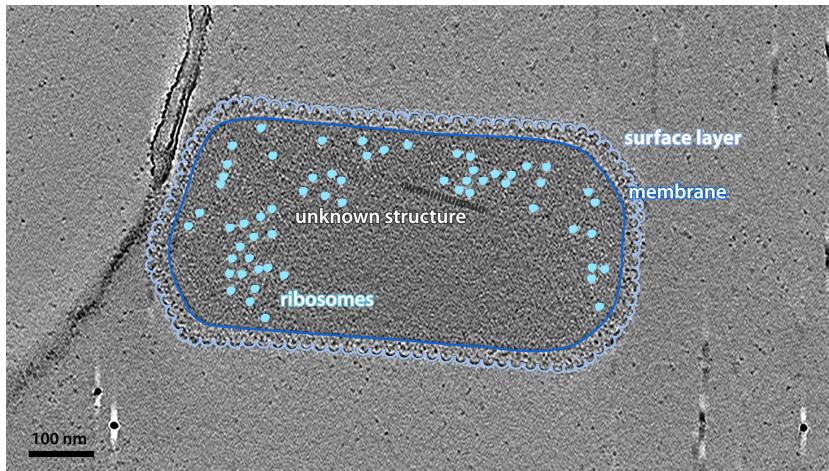


Figure 2.15: *Nitrosopumilus maritimus* Collected by: Rasika Ramdasi [10.22002/D1.1368](<https://doi.org/10.22002/D1.1368>)

2.7.3 *Methanoregula formicica*

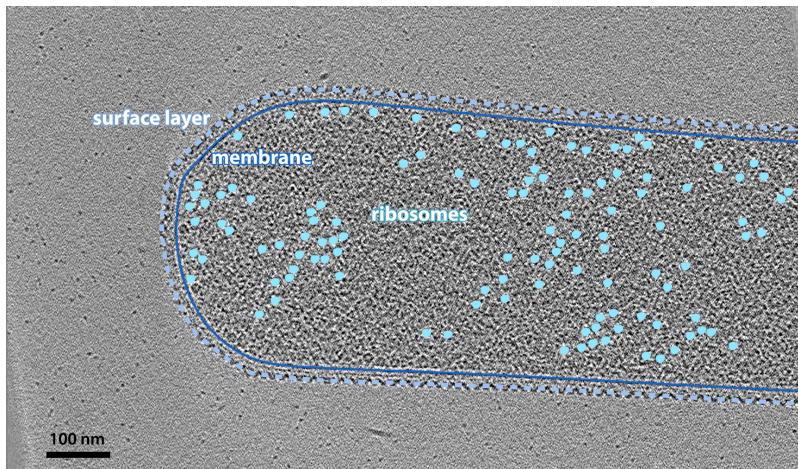


Figure 2.16: *Methanoregula formicica* Collected by: Ariane Briegel [10.22002/D1.1369](<https://doi.org/10.22002/D1.1369>)

2.8 *Methanospirillum hungatei*

Why stop with a single layer of protein? For proof that Nature is endlessly inventive, consider this *Methanospirillum hungatei* cell. These archaea encase themselves in an S-layer, and then an additional protein layer that forms a highly impermeable sheath. The sheath is also very resistant to pressure, which could be important in the cells' line of work. *M. hungatei* were discovered in sewage, where they break down organic waste, producing methane. One theory is that the sheath acts as a pressure regulator; when enough methane builds up inside the cell, the pressure expands the sheath, opening its pores

wide enough to allow the methane to dissipate and new metabolic substrates like hydrogen and carbon dioxide to enter.

The rules of architecture remain the same, though. Just as in the bacterial cell wall, sheath polymers are arranged as hoops perpendicular to the long axis of the rod-shaped cell. The ends of the sheath, as you can see, are special, with multiple protein layers stacking into a thick plug. Cells divide within the sheath, and long chains of cells in a continuous sheath are often observed.

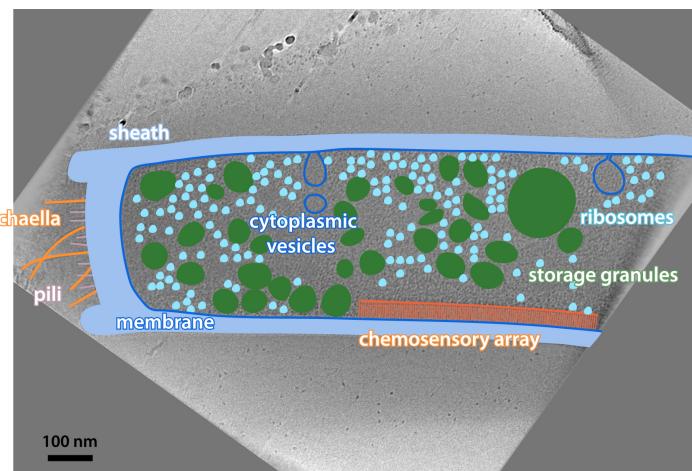


Figure 2.17: *Methanospirillum hungatei* Collected by: Ariane Briegel [10.22002/D1.1357] (<https://doi.org/10.22002/D1.1357>)

2.9 *Haloarcula argentinensis*

All the layers we've just discussed collectively make up the container, or envelope, of a cell. As you've seen, different species use different combinations of these components to form their envelopes; the only constant is the cytoplasmic (or inner, for diderms) membrane. Before we move on, let's talk briefly about what these envelopes contain. In addition to water and small molecules, you've already seen some large protein complexes like motility machineries. You've also seen the ribosomes – the protein/RNA complexes responsible for translation. But you might have been surprised not to see something else: DNA. The replicating molecule containing the instructions for the life of the cell is of paramount importance but often invisible by microscopy. But not always.

Thin filaments of DNA, only about 2 nm wide, blend in with the dense cytoplasm of the cell. When a cell lyses, though, its cytoplasm diffuses into the environment and the DNA filaments stand out against the now-much-reduced background. You can get an idea of the sheer abundance of DNA in a cell from this *Haloarcula argentinensis* where the envelope has ruptured and the contents are spilling out.

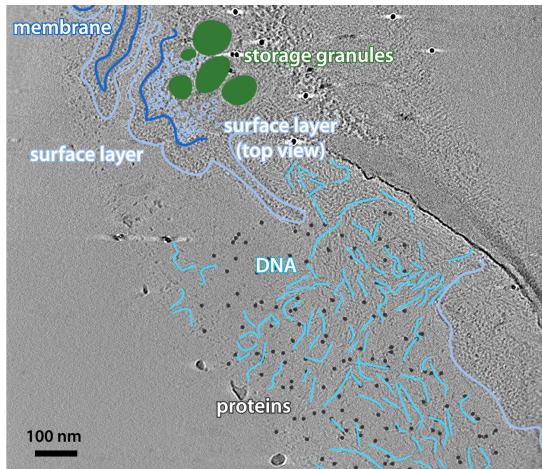


Figure 2.18: *Haloarcula argentinensis* Collected by: Ariane Briegel [10.22002/D1.1358] (<https://doi.org/10.22002/D1.1358>)

2.10 *Bdellovibrio bacteriovorus*

Cells contain enormous amounts of DNA. The single, circular chromosome of this *Bdellovibrio bacteriovorus* cell contains 3,782,950 individual base pairs, which means that if the circle were cut and laid out as a long piece, it would be about one thousand times longer than the cell itself. To fit and function inside the cell, the chromosome has to be extraordinarily organized and packed, a feat we still don't understand. You can see some of this packing in nearly every cell: the center of the cell tends to have very few large macromolecular complexes like ribosomes, because they're excluded by the densely-packed chromosome(s). Watch out for these ribosome-excluding zones in the rest of the book; they indicate the location of the bulk of the cell's DNA. Since bacteria and archaea don't enclose their DNA in a subcellular membrane, we don't call this region a nucleus (the "karyon" that defines eukaryotes). Instead, we use the term nucleoid to describe the cytoplasmic region where most of the DNA is concentrated.

At times, the nucleoid becomes easier to see. Imagine that you want to decrease gene expression in your cell. Don't worry yet about why – we'll discuss that in Chapter 8 – for now, just think about how. One approach is simply to pack the chromosome so tightly that the transcriptional machinery can't access the genes. That's what this cell is doing, condensing its nucleoid into a dense twisted braid we can easily visualize.

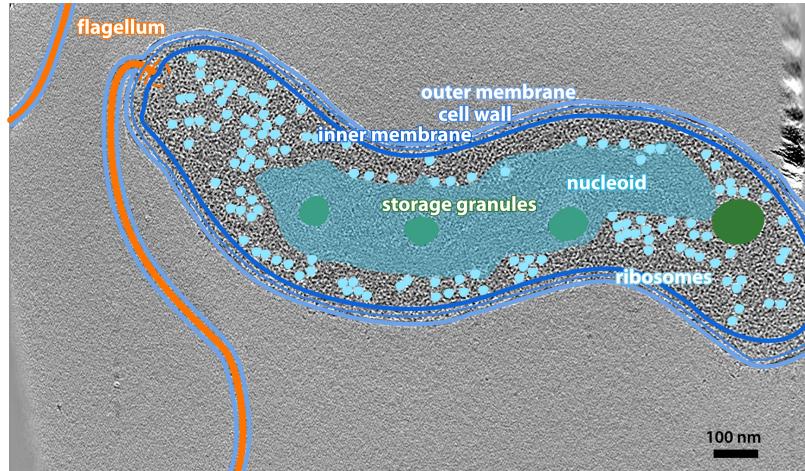


Figure 2.19: Bdellovibrio bacteriovorus Collected by: Yi-Wei Chang
[10.22002/D1.1359](<https://doi.org/10.22002/D1.1359>)

3

Shape

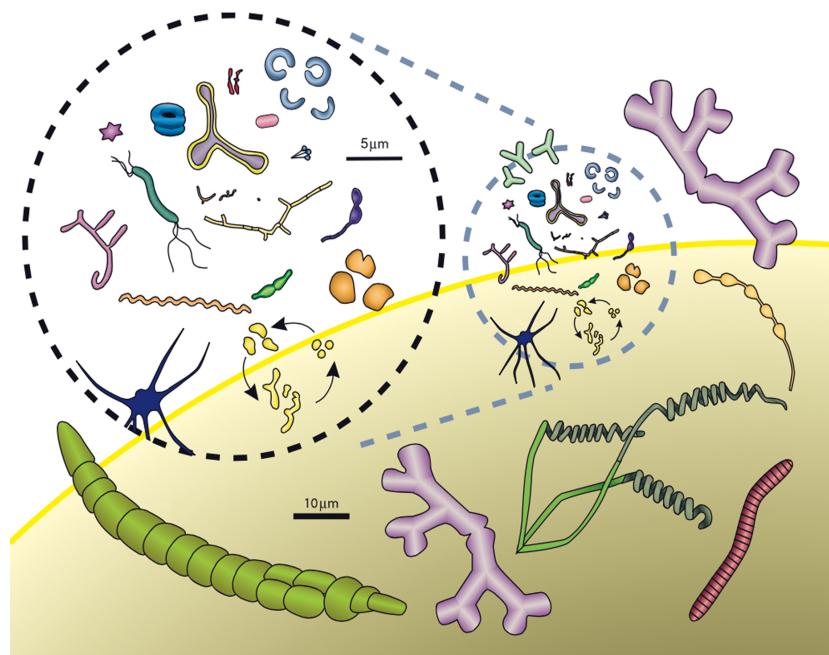
3.1 *Simkania negevensis*

What kind of life do you envision for your cell? Just as the design of buildings reflects their purpose, different cell shapes suit different lifestyles. Does your cell need to soak up sunlight for photosynthesis? Burrow through tissue? Chase down prey? Each driving function is best served by a particular form Schematic – Cell shape diversity.

How can you give your cell a particular form? The final shape of a building is determined by a façade erected around a system of internal beams and joists–its skeleton. Cells determine their shape using a similar system—the cyto-(“cell”) skeleton—in concert with the rigid exoskeleton of the sacculus. The cytoskeleton of bacteria and archaea consists of a set of proteins that form filaments or other superstructures that move or scaffold other material in the cell. In many cases, this cytoskeletal scaffolding is dynamic and ever-changing, appropriate for a living building.

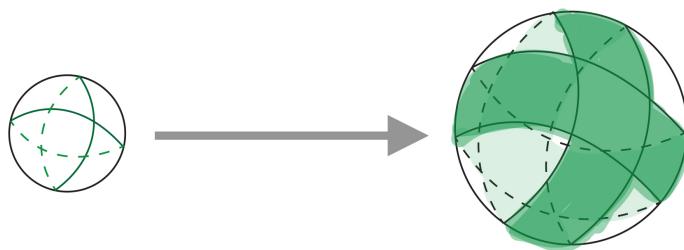
Let’s start with a cell like this *Simkania negevensis*. It’s a sphere—the default shape for a membrane in water, uniformly resistant to pressure, and the best shape if you want to maximize your volume relative to surface area. We refer to this spherical shape as coccoid (“berry-like”). To grow, a coccoid cell can just randomly insert new glycan strands into its sacculus, expanding into a larger sphere Schematic – Spherical growth.

3.1.1 Cell shape diversity



Archaea and bacteria adopt a staggeringly diverse set of shapes. Here are some examples just of bacteria. Note also the range of sizes; what might at first look like a hill at the bottom is the edge of a cell drawn to scale.

3.1.2 Spherical growth



3.2 *Cupriavidus necator*

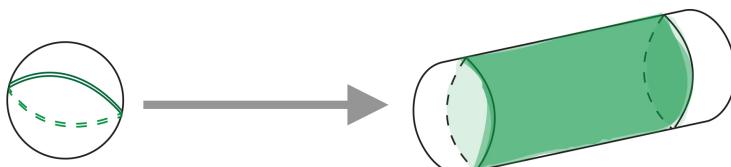
Instead of a sphere, what if you wanted to make your cell cylindrical, like this *Cupriavidus necator*? So-called rod-shaped cells (cylinders with spherical caps) are a very common form for bacteria and archaea, likely because they're efficient swimmers and swarmers (more on that in Chapter 6). Starting from a sphere, imagine that you had a construction contractor who could direct where workers lay in new cell

wall. Instead of random insertion, you could, say, direct them to work around a single plane. As the workers laid in more and more hoops of peptidoglycan in this region, a cylinder would form with the same diameter as the initial sphere (which would now serve as the structure's end caps) Schematic – Rod-shaped growth.

The contractor for most rod-shaped bacterial cells is a cytoskeletal protein named MreB (the name reflects the discovery of its gene neighborhood as a region in which mutations gave *E. coli* resistance to the antibiotic mecillinam). MreB is a homolog of the eukaryotic cytoskeletal protein actin. It's still not clear exactly how it works (cryo-ET debunked a once-leading theory), but small patches of MreB seem to shuttle rapidly around the circumference of the cell, directing the proteins you saw in the last chapter to add new peptidoglycan to the sacculus. MreB's circuit is restricted to the cylindrical portion of the cell, expanding the cylinder without affecting the ends. This growth pattern has an interesting consequence: the peptidoglycan in the cell caps is older than the peptidoglycan in the cylindrical center. The two types, old and new, can thus serve as convenient landmarks, allowing the cell, for instance, to tether something to the end. We'll discuss how that can be useful in Chapter 5.

Not all rod-shaped bacteria use MreB, and we're still figuring out how the shape forms in many species [More – Rod variety]. For rod-shaped archaea [More – Archaeal rods], the S-layer is important for determining shape, but the mechanisms remain unclear.

3.2.1 Rod-shaped growth



3.2.2 *Brucella abortus*

Rod-shaped species aren't always perfect rods. For instance, they might be more pear-shaped, like this *Brucella abortus* cell. This is one of the bacterial species that doesn't use MreB.

3.2.3 *Methanoregula formicica*

3.3 *Hylemonella gracilis*

Rod-shaped cells have a useful property: they can grow by extending their length without significantly changing the ratio of their surface area to volume, which would in turn change how efficiently they can take up nutrients from the environment. This property enables an impressive range of lengths for rod-shaped cells, from the short C. necator you just saw, to this much longer *Hylemonella gracilis*.

The length of a cell (or, more generally, its size) varies depending on the environment or its stage of the lifecycle. But not that much. Size tends to be strongly conserved within a species, varying not much more than the factor of two dictated by division. Sizes between species vary much more widely, as you'll see throughout the book.

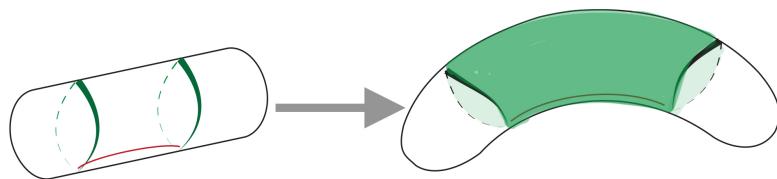
3.4 *Caulobacter crescentus*

What if you want to curve your rod-shaped cell into a comma? Vibrioid shape (named for the genus *Vibrio*, where it is common) may help cells swim faster. For *Caulobacter crescentus* like this one, it can help them stay close to a surface as liquid flows past, increasing the chance that their progeny can attach before they're swept away. To make a vibrioid sacculus, you can imagine the contractor simply telling the workers to incorporate more material on one side of the rod relative to the other Schematic – Vibrioid growth. This is the function of a cytoskeletal protein called (for an obvious reason) Crescentin. Crescentin inhibits cell wall synthesis. It also localizes to one side of the cell, resulting in more peptidoglycan incorporation on the opposite side, and a curved cell.

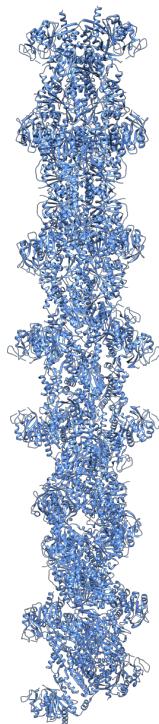
Checks and balances are a common theme in Nature. In this case, a second contractor makes sure the process doesn't get out of hand. The cytoskeletal protein CTP synthase inhibits Crescentin, fine-tuning the process and making sure the cell doesn't curve too much. As its name implies, CTP synthase has another, metabolic, function in the cell Schematic – CTP synthase. In *C. crescentus* like this, the CTP synthase is easy to see as a bundle of filaments. Crescentin is more elusive; we only see obvious filaments when it is artificially overexpressed, so its exact form in the cell remains unclear.

This system is one way of making a curved cell. There must be others, though, since many vibrioid species (including *Vibrio*!) don't use Crescentin. As you'll see throughout this book, there is no shortage of biological questions still to figure out.

3.4.1 Vibrioid growth



3.4.2 CTP synthase



CTP synthase is a fundamental metabolic protein across all domains of life, helping make the building blocks of RNA and DNA. It is also not the only metabolic protein seen to form filaments in bacteria and archaea. This suggests that polymerization might be serving another role, perhaps as a way to regulate the activity of an enzyme that may not always be needed, but would be costly or slow to degrade and synthesize again. In that case, the cytoskeletal role may have arisen secondarily; once you have a long filament lying around, why not use it as scaffolding?

3.5 *Campylobacter jejuni*

Why stop at a quarter turn when you can twist your cell into a full wave or even a corkscrew? Just as the corkscrew penetrates its target, helical pathogenic bacteria like this *Campylobacter jejuni* can burrow efficiently into the tissue of their target. More on that in Chapter 6. For now, let's just consider how these shapes can be formed.

It's tempting to group species with a common characteristic, but appearances are often deceiving about relatedness. Helical shape, for instance, was not a one-shot invention; it was invented independently multiple times. This is true of other bacterial and archaeal cell shapes as well. For helical shape, these independent origins are reflected in diverse mechanisms of creating it. Some species, like *C. jejuni*, use dedicated proteins to regulate the pattern of peptidoglycan insertion—a continuation of the theme we've been discussing. Other species take different approaches [More – *Borrelia burgdorferi*].

3.5.1 *Borrelia burgdorferi*

Borrelia burgdorferi like this cell use the long filaments of their motility machinery (flagella, discussed in Chapter 6) as a kind of cytoskeleton. A bundle of flagella wraps around the cell in the periplasm, between the sacculus and the outer membrane. As motors at their base spin them, these filaments impart a helical wave pattern to the sacculus; without their rotation the cells develop a rod shape. *B. burgdorferi* aren't actually helical; rather they are wave-shaped, a 2-dimensional curve instead of a 3-dimensional one.

3.6 *Verrucomicrobium spinosum*

Motility isn't everything. Another major force shaping cells is metabolism. Nutrients are often scarce, and increasing your cell's ability to absorb them can give it a boost in the competitive game of life. So how can you do that? Remember that a sphere maximizes volume relative to surface area. To maximize surface area (for nutrient uptake) relative to volume, you'd want something spikier. Some bacteria extend prosthecae ("add-ons" or appendages) for this purpose. Some, like *Caulobacter crescentus*, use a single prostheca, which is also called a stalk. Stalks are commonly located at the end of the cell, where, as you'll see in Chapter 8, they can help attach the cell to a surface, letting them hang on even in turbulent flow in the freshwater lakes and streams where they live. Other species have a stalk on either end. Still others, like this *Verrucomicrobium spinosum*, form astral shapes with prosthecae jutting out in all directions.

Prosthecae offer a challenge for the architect: thin extensions are

delicate. Prosthecate cells use cytoskeletal proteins to form and stabilize their stalks, although exactly how this works remains unclear. One of these cytoskeletal proteins is Bactofilin Schematic – Bactofilin, which is similar to the proteins that make intermediate filaments in eukaryotes. *C. crescentus* use Bactofilin polymers to help make their stalks. Prosthecobacter contain microtubules [More – Bacterial microtubules] in their stalks, but their function remains unclear.

3.6.1 *Bactofilin*



3.6.2 *Prosthecobacter vanneervenii*

Some bacterial species with prosthecae express structures similar to eukaryotic microtubules, made from two proteins called BtubA and BtubB to reflect their homology to eukaryotic tubulins. Eukaryotic microtubules are hollow tubes formed by 13 protofilaments; bacterial microtubules are smaller, with only ~5 protofilaments. Cells commonly contain a bundle of microtubules in their prostheca, like this Prosthecobacter vanneervenii cell, which has a bundle of four.

Prosthecobacter belong to an evolutionarily unique group of species that share characteristics unusual in the rest of the bacterial phylogenetic tree. We refer to the collective group as the PVC superphylum (because it contains Planctomycetes, Verrucomicrobia, and Chlamydiae). Having homologs of eukaryotic microtubule proteins is one of these unique characteristics; so far, Btubs have only been identified in species of Prosthecobacter. They seem to have come from a horizontal gene transfer from a eukaryotic cell (meaning that microtubules evolved first in eukaryotes and were later borrowed by the bacteria).

3.7 *Haloquadratum walsbyi*

All the cells whose shape we've discussed so far have been bacteria, but archaea hold their own in the specialized shape competition. In

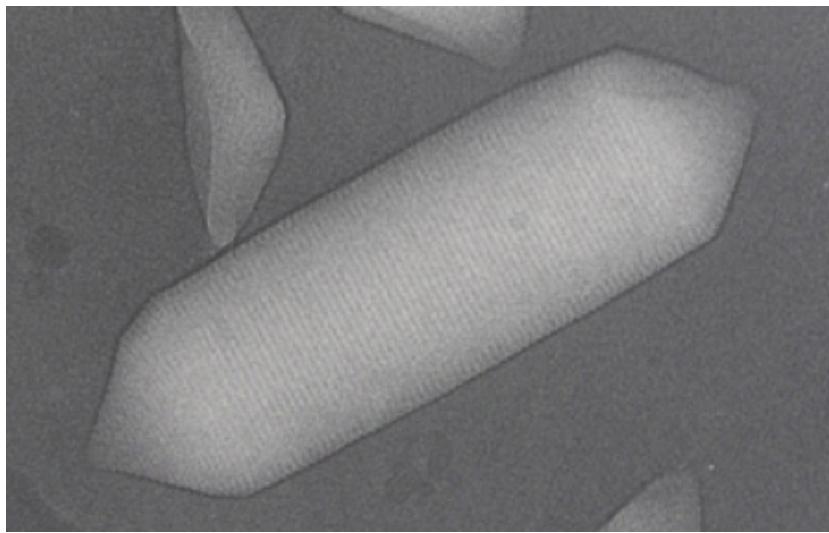
fact, one of the most extreme examples of maximizing surface area relative to volume comes from this archaeon, *Haloquadratum walsbyi*, which grows as thin, square tiles. Very thin, square tiles. This property helps keep them oriented with their broad sides to the sun, whose light they convert to energy. They divide in this plane, too, with their progeny extending the sheet of tiles. Gas vesicles [More – Gas vesicles] keep the cells buoyant in the super-salty lakes where they live.

It's still not exactly clear how this shape is determined, but at least part of the mechanism seems to involve glycoproteins on the cell surface.

3.7.1 *Halobacterium salinarum*

Some species of archaea and bacteria use gas vesicles to control their buoyancy. This can allow them to rise or fall in a water column, which can be a great advantage. *Halobacterium salinarum* like this one produce gas vesicles in response to cues from the environment, lifting themselves out of the sediment and into more favorable conditions of oxygen or sunlight for photosynthesis. This cell has just started producing gas vesicles, so they are small and isolated. Later, the vesicles will elongate into cylinders with conical ends Schematic – Gas vesicles. Each cell might contain dozens of vesicles, and they often cluster together.

3.7.2 Gas vesicles



Gas vesicles are microcompartments enclosed by a hydrophobic shell made of a single layer of protein. (Sometimes additional proteins reinforce the shell.) Gas vesicles don't actually store gas; they simply allow gas dissolved in the cytoplasm to diffuse in, while forming

a tight barrier against anything else, like water. They are sensitive and prone to collapse with even a slight increase in the surrounding pressure.

4

Growth

4.1 *Caulobacter crescentus*

The life of a cell is simple in theory, and complex in practice. Think about the evolutionary purpose of your cell. Fundamentally, it is to grow, amassing enough resources to eventually multiply itself. How best to grow, though, depends on the environment and what's available to use as fuel. Different species have evolved different strategies to optimize their growth. In a world of scarce resources and fierce competition, any adaptation that lets your cell grow more efficiently can have a big effect on its success. Often these adaptations are visible in the structure of the cell, as you'll see in this chapter.

You've already seen how the shape of your cell can allow it to gather nutrients from the environment more efficiently. For example, *Haloquadratum walsbyi* uses a flat shape to maximize its exposure to sunlight for photosynthesis. Alternatively, prosthecate bacteria like *Caulobacter crescentus* use long, thin extensions to increase surface area relative to volume, allowing them to absorb more nutrients. Let's examine these extensions more closely.

The extra surface area of a stalk can increase your cell's ability to absorb nutrients, but it also adds a lot of membrane that can dilute membrane proteins and increase the time it takes them to diffuse around the cell. *C. crescentus* stalks get longer throughout their lifetime, so the problem only gets worse with age. As an engineer, how could you solve this problem? What if you could separate the envelope of the stalk from the envelope of the rest of the cell? *C. crescentus* cells like this one have evolved an elegant way of doing this: stalk bands. These protein structures form diffusion barriers for the membranes and periplasm, but not the cytoplasm. Each cell division produces another band in the elongating stalk, so you can tell a cell's age by counting its bands.

[2_Shewanella oneidensis]

Other cell extensions also help metabolism. Non-photosynthetic

cells break down nutrients into chemical energy (ATP) through respiration reactions. The chemistry is beyond the scope of this book, but it involves the transfer of electrons to an acceptor molecule, typically oxygen (the “aer” in aerobic respiration). When oxygen isn’t present, some cells can use an alternative electron acceptor, such as iron or sulfur, in a process called anaerobic respiration. This works well when the acceptor is soluble and can diffuse into the cell. But what if the only available acceptor is trapped in a mineral? *Shewanella oneidensis* like this one have evolved a workaround: they extend outer membrane vesicle chains. Electron carriers in the membrane Schematic – Cytochromes shuttle electrons to metal oxide minerals in the environment, a conductive property that lends the appendages the name “nanowires.” The morphology of *S. oneidensis* nanowires is similar to that of other outer membrane vesicle chains, with tubular and pearly sections. Here the nanowire has detached from the cell. You can also see metal deposits on the cell’s envelope, which it is presumably using for respiration.

Other species, like *Geobacter sulfurreducens*, also use nanowires to transfer electrons to an insoluble acceptor in the environment, but the structure of these nanowires is very different: a long filament made from stacked cytochrome proteins, resembling a pilus (thin protein filaments you’ll see in Chapters 6 and 9).

4_2_1 Schematic: Cytochromes

This model shows the arrangement of cytochromes (red and green dots) on the section of *S. oneidensis* nanowire you saw on the main page. The membrane is colored in gold.

4.2 *Rhodopseudomonas palustris*

What if your cell gathers energy from sunlight; how can you make that process more efficient? Photosynthetic bacteria harvest light energy using protein complexes Schematic – Photocomplexes embedded in their membranes. It seems intuitive to increase the light-gathering capability of such a cell by adding more membrane. But then the cell’s volume would also increase. How could you get around this problem? Why not stack the extra membrane in the cytoplasm? Many (but not all) photosynthetic bacteria contain intracytoplasmic membrane, or ICM. In cells like this *Rhodopseudomonas palustris*, the ICM is continuous with the inner membrane and forms stacks at the middle of the cell. Different species can have different, and more extensive, arrangements [More – ICM variety].

4_3_1 Schematic: Photocomplexes In the ICM, the light-harvesting photocomplexes are themselves highly organized. As you can see here, the complexes comprise hundreds of individual proteins and can reach

tens of nanometers in size. Physically tethering enzymes that function in the same pathway increases efficiency by allowing the enzymes that catalyze subsequent steps to hand off substrates. You'll see more examples of this theme in the rest of the chapter; metabolic enzymes in the cell are often clustered together into relay teams.

4.2.1 *Methyloprofundus sedimenti*

In some species, like this *Methyloprofundus sedimenti*, the ICM is even more extensive and appears to be fully separated from the cell membrane. As you can see, it occupies much of the cytoplasmic space.

4.3 *Chloroflexi*

Another approach to accommodate additional enzymatic workers is to put them in a dedicated factory. Rather than extending their membranes, some bacteria, like this *Chloroflexi*, use dedicated structures called chlorosomes to pack together many light-harvesting complexes. This approach of bringing together enzymes that function in the same metabolic pathway is a common way cells increase efficiency.

4.4 *Thiomonas intermedia*

You might be able to increase the efficiency of your factory even further by enclosing it in a dedicated building. Many bacteria (but not, as far as we know, archaea) do this by forming microcompartments—areas of the cytoplasm walled off by a protein shell Schematic – Microcompartment shells. One of the most impressive microcompartments is the carboxysome that some bacterial species use for carbon fixation (the breakdown of carbon dioxide into usable fuel molecules). Carboxysomes contain tightly packed copies of an enzyme called ribulose bisphosphate carboxylase/oxygenase, or more simply, RuBisCO, which, incidentally, is the most abundant enzyme on Earth. The shell does more than simply concentrate the enzymes. Oxygen competes with carbon dioxide for binding RuBisCO, so the bacteria include another enzyme in the compartment which converts bicarbonate into CO₂, making it readily available to the RuBisCO. The shell may also slow down the diffusion of CO₂ out, and O₂ in. Carboxysomes probably weren't necessary in early cells because the oxygen level of the environment was much lower. Cells like this *Thiomonas intermedia* contain many carboxysomes. While most are icosahedral, there is variation in their forms [More – Carboxysome variety].

Microcompartments can protect their contents from the rest of the cell, or they can do the reverse. Some metabolic pathways generate

toxic intermediates, so bacteria have evolved microcompartments to sequester them so they don't interfere with other processes in the cell.

4_5_1 Schematic: Microcompartment shells

Bacterial microcompartments are typically enclosed by a self-assembling shell formed by just a few proteins. The shell is icosahedral, consisting of hexameric units packed into flat planes, which are joined together by pentamers at the vertices. You can see this arrangement in the shell structure of a microcompartment called an encapsulin that helps *Thermotoga maritima* respond to stress.

4.4.1 *Halothiobacillus neapolitanus*

Most carboxysomes look like the ones you saw on the last page, but some look different. Some are less regular polyhedra, as in this *Halothiobacillus neapolitanus* cell. And some are stranger still [More – Long carboxysomes].

4.4.2 *Hydrogenovibrio crunogenus*

These carboxysomes in a *Hydrogenovibrio crunogenus* cell have grown abnormally long, extending across its width. These are unusual, but more minor elongations are common.

This cell also highlights the lability in the outer membrane common in some bacteria, particularly pathogens. Note how the cell has curled inside its loose outer membrane. You can also see it stretching across to another cell in a neighboring hole; they were probably just finishing division when they got pulled apart.

4.5 *Cupriavidus necator*

No matter how efficient your factories are, they need raw materials, and in an ever-changing environment, your cell can't always depend upon a steady supply chain. How can you help your cell cope with occasional shortages? If you said stockpile, you're in good agreement with Nature. Both bacteria and archaea use storage granules to stockpile essential nutrients. The substrate is usually polymerized for easier packing, like poly-phosphate or the poly-hydroxybutyrate in these granules in *Ralstonia eutropha*. No matter what they pack, storage granules are generally spherical, and exhibit a range of sizes [More – Storage granule growth]. When the environment is rich in one nutrient but poor in another, cells may stop growing, but keep adding to their cellular stores. You can see that effect in this *C. necator* cell which has been cultured in a medium with carbon but not enough nitrogen; as a result, it has accumulated very large poly-hydroxybutyrate granules which take up much of the cytoplasmic space.

Storage granules are ubiquitous in bacteria, and you'll see them in many of the cells in this book. The most common type is poly-phosphate, which has a characteristic dark appearance in electron microscopy images.

4.5.1 *Lysobacter antibioticus*

Storage granules expand and contract to accommodate their contents. This *Lysobacter antibioticus* cell exhibits a typical range of sizes of poly-phosphate granules.

4.6 *Agrobacterium tumefaciens*

Some storage granules, like the poly-phosphate and poly-hydroxybutyrate granules you just saw, are very densely packed and clearly delineated from the rest of the cytoplasm. Other types are less tightly packed and more amorphous, like the ones in this *Agrobacterium tumefaciens* cell. We don't know yet what these contain.

In some species, like *Ralstonia eutropha*, storage granules seem to be positioned randomly in the cell. Other species have more regulated arrangements. *A. tumefaciens* cells always have one poly-phosphate storage granule located near the cell pole. Other species have one at each end of the cell, as you'll see on the next page. As you'll see in Chapter 5, this arrangement can help mother cells pass on a storage granule to each of their daughters during division.

4.7 *Hydrogenovibrio crunogenus*

There seems to be a relationship between bacterial microcompartments and storage granules. For instance, small poly-phosphate storage granules are sometimes seen inside carboxysomes, as in this *Hydrogenovibrio crunogenus* cell.

4.8 *Halothiobacillus neapolitanus*

We also sometimes see comb-like structures connecting external poly-phosphate storage granules with carboxysomes, as in this *Halothiobacillus neapolitanus* cell. The nature of the relationship between the structures, and the identity and function of the combs, remains a mystery.

4.9 *Halorubrum litoreum*

Archaea also use storage granules, and you'll see many examples throughout the book. Compared to the smooth edges of bacterial

granules, the edges of archaeal granules are spikier, giving them a rougher appearance, as in this *Halorubrum litoreum* cell.

5

Division

5.1 Thiomonas intermedia

Growth only gets you so far. If your cell is coccoid, growth increases volume more rapidly than surface area, which is a problem if your cell relies on nutrients imported from the environment. Even if your cell is rod-shaped (so its surface area to volume ratio remains relatively constant), increased volume increases diffusion times, making metabolism less efficient. So what can you do to keep your cell thriving?

It may be time to divide. In essence, division simply splits a cell, the “mother,” into two “daughters.” Each daughter will be roughly half the size of the mother. A fair split, though, particularly of critical components like the genome, requires careful coordination. Think about the things in your cell. Some of them are present in many copies, like most of the proteins. Others are present in very few copies, like the chromosome(s). Conceptually, we can sort components into two broad categories: high copy-number and low copy-number items. How would you split the high copy-number items? Easy, right? Just split the cell in the middle and each half will have plenty. This is true, for instance, for the ribosomes and carboxysomes you see in this *Thiomonas intermedia* cell.

What about a low copy-number component? Look at the poly-phosphate storage granules in the same cell. To ensure each daughter cell gets the same number, they’re evenly spaced along the length of the cell, ready for division in the middle.

5.2 Caulobacter crescentus

The most important low-copy number component in your cell is its genome; without the instructions, nothing gets built. Reflecting its importance, cells have evolved complex mechanisms to coordinate DNA replication and segregation in time as well as space. The details are beyond our scope here, but let’s examine some general structural prin-

ciples. If your cell divides by splitting in the middle, what's the easiest way to get one complete genome copy to each daughter? Assume your cell has a single chromosome (like most bacteria and archaea). Why not just tether each chromosome copy to an opposite pole of the cell? Nearly all bacteria, and many archaea, have a protein called ParB (for Partitioning) that recognizes a specific sequence (ParS) on the chromosome, creating a molecular handle. In *Caulobacter crescentus* like this cell, ParB also binds a scaffolding protein at the pole called PopZ, hooking the handle to the pole. The PopZ scaffold isn't highly ordered, so we see it as a diffuse blob of DNA and protein, noticeable because it excludes other large protein complexes like ribosomes. Several species of bacteria use PopZ or other hub-organizing proteins to tether a genome copy, as well as other things like chemosensory machinery, to the cell pole. Other species use a different mechanism involving many copies of a dynamic protein called ParA that bind and release ParB, ratcheting the ParS handle of the chromosome across the cell. Other components, like the storage granules you just saw, piggyback on this machinery for segregation.

Remember that your cell's chromosome is colossal, so getting the ParS handle to one side is only part of the battle. An army of other proteins work to condense the chromosome to a more manageable volume and wrangle with the division machinery to make sure no straggling loops get caught off-sides.

5.3 *Hyphomonas neptunium*

What if your cell divides a different way? Some bacteria divide not by fission, but by budding, like this *Hyphomonas neptunium* cell. These cells have a stalk [More – *Hyphomonas* stalk] and concentrate their growth at the end of the stalk, producing a daughter cell like blowing a bubble. When the bud becomes big enough, they divide at the end of the stalk to release it [More – *Hyphomonas* life cycle]. First, though, they have to make sure all the necessary components make it into the bud. The process is most dramatic for the genome; here you can see a copy being transferred through the stalk. The chromosome here resembles a single double-stranded DNA helix, but it's actually a higher-order structure of supercoiled DNA. (The crossbands aren't hydrogen-bonded bases, but rather proteins that help pack the DNA.)

Several other bacterial species divide by budding, although not all have stalks; some simply bud from the main cell body, as you'll see later in this chapter.

5.3.1 *Hyphomonas neptunium*

Hyphomonas neptunium grow a single stalk from one end of their cell body, similar to the Caulobacter crescentus you saw in Chapter 3. The function of this stalk, though, is very different, as you'll see.

5.3.2 *Hyphomonas neptunium*

Hyphomonas neptunium have evolved a program of stages they pass through in the course of their life. When a newborn cell like this one is released, it is in the “swarmer” stage, using its flagellum (discussed in the next chapter) to swim away to a new location. After a while, it settles down, jettisoning its flagellum and growing a stalk to ultimately make its own bud—the next generation. Once a cell settles down into the stalked stage, it spends the rest of its life sending off buds as long as conditions are good. Only new cells are equipped with flagella to go adventuring. We'll discuss how lifecycles like this can be a beneficial strategy in Chapter 8.

[4_Staphylococcus aureus]

Once your cell has gotten everything where it needs to go, how can it actually divide? Fences separate neighbors, so why not use the cell wall to build a septum (or “fence”) between the two daughters? That's what this Staphylococcus aureus cell is doing. In monoderm bacteria like this with a thick cell wall, it's easy to see the septum.

5.4 *Tetrasphaera remsis*

The septum grows in from the periphery of the dividing cell toward the middle, drawing the membrane with it. When it reaches the middle, the membrane seals off on each side and the septum separates to release the two cells. The Tetrasphaera remsis cell at the bottom is at a fairly late stage of this process. This cell also illustrates another point about division: the division plane isn't always exactly in the middle of the cell. Depending on the species, the two daughter cells may be different sizes.

There can also be more than two daughter cells. Some species undergo simultaneous divisions, with two or more septa forming more or less at the same time. The genus of Tetrasphaera remsis gets its name from this property—cells can either divide into two (as in this case) or four, roughly spherical cells.

5.5 *Idiomarina loihiensis*

The division process is similar for diderm bacteria, as you can see in this Idiomarina loihiensis cell, with the cell wall and inner membrane

building inward. The process is almost complete here, with just a thin channel of cytoplasm connecting the daughter cells in the very middle. You can see the cell wall zippering apart at the edges of the septum. For another example slightly earlier in the process, check out [More–*Sphingopyxis alaskensis* division]. For a later example, check out [More–*Chloroflexi*]. In some species, the outer membrane constricts at the same time as the inner membrane, as you saw, for instance, in the *T. intermedia* cell at the beginning of this chapter. In others, including this one and *S. alaskensis*, the outer membrane is mainly remodeled at the end of the process, as the cells separate. Other species are intermediate [More–*Caulobacter crescentus* division].

5.5.1 Sphingopyxis alaskensis {#*Sphingopyxis_alaskensis_division*} This diderm cell is about halfway through division. The two daughter cells are still connected by a wide bridge of cytoplasm, but the inner membrane and cell wall are drawing in all around.

5.5.2 Chloroflexi

This diderm cell is in the final stage of division. The cytoplasm of the two daughter cells is completely separated by inner membrane and cell wall. The outer membrane, though, has not yet divided.

5.6 Caulobacter crescentus

In some species, the outer membrane constricts at the same time as the inner membrane, as you saw, for instance, in the *T. intermedia* cell at the beginning of this chapter. In others, including the cells you saw on the last page, the outer membrane is mainly remodeled at the end of the process, as the cells separate. Other species are intermediate. In *Caulobacter crescentus*, the outer membrane constricts along with the inner membrane and cell wall. At the end of division, though, the inner membrane seals off before the outer membrane, as you can see in this cell, which is at the very end stage of division. For an even later stage, check out [More–*Caulobacter* division].

5.6.1 Caulobacter crescentus

These daughter cells have completely separated their mother's inner membrane and cell wall, but not yet the outer membrane and surface layer.

5.7 *Agrobacterium tumefaciens*

Like monoderms, most diderms divide into roughly equally-sized daughter cells, but not all do. Just like the *Tetrasphaera remsis* you saw earlier, some diderm species produce one larger, and one smaller, daughter, like this *Agrobacterium tumefaciens* cell. In this case, it's because the smaller daughter is actually a bud. *A. tumefaciens* concentrate their growth on one pole, pushing out a bud that, once it's large enough, is pinched off and released. The process is very similar to what you saw in *Hyphomonas neptunium*, just without the stalk.

5.8 *Cupriavidus necator*

Division can also be asymmetric in a different way: how constricted one side of the cell is relative to the other. You can see that in this *Cupriavidus necator* cell. Asymmetric constriction is most common early in division, but sometimes it persists for a while [More-Asymmetric constriction]. To understand how this happens, let's take a closer look at how your cell divides.

To divide, your cell's membrane and cell wall constrict. But remember that the cell wall is resisting a lot of force coming from turgor pressure pushing outward. So how can it overcome this pressure and grow inward? For almost all bacteria and many archaea, the answer is a protein contractor called FtsZ (Filamenting Temperature-Sensitive mutant Z, named for a genetic screen for cells that didn't divide, and therefore grew very long, or filamentous). FtsZ is another piece of your cell's cytoskeleton. Homologous to eukaryotic tubulin, FtsZ polymerizes into filaments that are linked to the cell membrane at the division plane [Schematic-FtsZ structure]. The filaments are highly dynamic, forming, disassembling and reassembling within seconds.

How does FtsZ find the right spot? There are multiple mechanisms, but an elegant one in cells like this one involves an inhibitor that localizes to the cell poles, making a repressive gradient that's strongest at the ends of the cell and weakest in the middle. As the rod-shaped cell grows, the inhibitor concentration at the middle eventually drops low enough that FtsZ can polymerize. Most cells also inhibit FtsZ polymerization when the genome is still in the middle, a mechanism called "nucleoid occlusion." Some species do this with an FtsZ-inhibiting protein called MipZ that grabs onto the ParB chromosomal handle, ensuring that FtsZ filaments don't form until the chromosome is clear.

Once everything's ready (the cell is long enough and the nucleoid's out of the way), FtsZ filaments form at the division site. Initially, a single, short filament (or a few) appears. Already, this is enough to

begin constricting the cell which explains why many bacterial cells, like this one, start to constrict only on one side. FtsZ filaments run parallel to the cell membrane, so they appear as dots in cross-section; but if we rotate the cell around, we can see them. Note: we can't see the membrane (and any associated FtsZ filaments) all the way around the cell due to the “missing wedge” of information in this imaging technique – see Chapter 1 for an explanation.

5.8.1 *Cupriavidus necator*

This *C. necator* cell is at a later stage of division. In most cells, as you'll see on the next page, constriction is more or less uniform around the circumference by now, but in some cells, including this one, the asymmetry persists.

5_9_1 Schematic: FtsZ structure

While the structure of FtsZ is known, its mechanism is not. It is an enzyme, so it's possible that the filament changes curvature as its sub-units flip from one conformation to another as a result of the chemical reaction (hydrolyzing GTP). This different filament conformation may pull on the membrane, allowing the cell wall to build inward.

5.9 *Caulobacter crescentus*

As division progresses, FtsZ filaments keep assembling and growing, and soon they form a complete ring of long, overlapping filaments all around the circumference of the cell. This drives further constriction, more or less uniformly. In this *Caulobacter crescentus* cell in a later stage of division, you can see many FtsZ filaments lined up in cross-section – the dots on both sides of the division plane; if we rotate the cell around, you can see filaments extending on both sides as far as the membrane is visible (see note on the previous page about why we can't see the membrane all the way around). They will continue to pull the membrane inward, and direct cell wall to be built behind it, until division is complete.

5.10 *Sulfolobus acidocaldarius*

Nearly all bacteria, and many archaea use FtsZ to divide. Other species of archaea, belonging to the Crenarchaeota phylum, use a different cytoskeletal system called CdV (for Cell division). CdV proteins are homologous to proteins of the Endosomal Sorting Complexes Required for Transport (ESCRT). ESCRT proteins were discovered in eukaryotes, where they are involved in many processes that involve cinching off membranes, from the final stage of cell division, to virus budding, to endocytosis (hence the name), where cells take things in

from the environment. Again, despite its fundamental importance, we still don't know exactly how the ESCRT machinery works to constrict membranes. In archaeal cells like this *Sulfolobus acidocaldarius*, Cdv proteins form a belt of parallel filaments around the middle of the cell, defining the division plane Schematic – ESCRT structure. Notice that the rigid surface layer is dismantled outside the belt so that the membrane can be pulled inward.

5_11_1 Schematic: ESCRT structure

5.11 *Sulfolobus acidocaldarius*

The Cdv filaments then pull the membrane inward, constricting the cell, as you can see in this *Sulfolobus acidocaldarius* cell in a later stage of division. Again, the mechanism isn't known, but it may be that the filaments slide against each other into a tightening spiral on either side Schematic – Hourglass model.

The majority of cells divide by one of these two mechanisms–FtsZ and Cdv—but not all do. Some bacterial and archaeal species have neither FtsZ nor Cdv, and we're still figuring out how they divide.

5_12_1 Schematic: Hourglass model

6

Motility

6.1 Caulobacter crescentus

Location, location, location. So far, we've been talking about how your cell can take the best advantage of its spot in the world. But why not find a better spot? Some cells live stationary lives, attached to a surface or embedded in a biofilm (more on these lifestyles in Chapters 8 and 9). But many cells are explorers, using an impressive variety of techniques to move through their environments, finding advantages in places with more food or fewer competitors. How would you go about making your cell a mobile home?

Most bacteria and archaea live in liquid, so motility means swimming. When you're the size of a cell, though, the backstroke won't get you very far. A rough measure called the Reynolds number describes the relative influence of inertia and viscosity on liquid flow, and this parameter scales with an organism's size. In the low-Reynolds number world of microbes, inertia is virtually nonexistent. When a rod-shaped bacterium stops swimming, it gets to coast only about the diameter of a hydrogen atom ($\sim 1\text{\AA}$) before stopping. In this molasses-like environment, rotary propellers work much better than paddles.

Nearly all bacteria that swim use the same propeller: a rotary motor embedded in their envelope that spins a long helical fiber called a flagellum [Schematic–Flagellum structure]. Flagella, like the one on this *Caulobacter crescentus*, are typically many times longer than the cell and take the form of a 3D wave [More–Flagellum morphology].

6_1_1 Schematic: Flagellum structure

The helical filament of the flagellum is made up of many copies of one or a few flagellin proteins. Each flagellin monomer has a soluble head domain and a hydrophobic alpha-helical tail that bundles together with the tails of other monomers to form the hollow tube. The tube comprises 11 protofilaments. [EMD-8847]

6.1.1 *Campylobacter jejuni*

Flagella are dynamic, as you'd expect. Throughout the book, you'll see examples caught in various conformations: straight, curved, or in a typical loose helical waveform as you see on this *Campylobacter jejuni* cell.

6.2 *Bdellovibrio bacteriovorus*

Let's take a closer look at the machinery of the motor that spins the flagellum. Broadly, we can break it down into a stationary part (the "stators") that anchors the machine in the cell and a rotating part (the "rotor") that spins the flagellum. A universal joint called the hook connects the filament to the machine's rotor. We can see these components more clearly by averaging individual motors from many cells to get a composite view [More–Flagellar motor structure].

The torque for spinning the flagellum comes from small movements in the stators that kick the rotor in a circle. The energy for these movements comes from the ion potential across the cell membrane that we discussed in Chapter 2; the stators provide a conduit for protons (in most species) or sodium ions (in some marine species) to diffuse down their chemical gradient into the cytoplasm, powering a conformational change in the stators in the process. The energy demands of the machine are high: a single rotation requires about 1,000 protons to flow through the stators, and the motor may spin at more than 6,000 rotations per minute. The fact that cells pay this energetic cost indicates a strong evolutionary selection for motility, or in other words, the powerful advantage your cell can gain by learning to swim.

While the basic plan of the motor is the same in every species with flagella, there are structural differences that reflect the different environments species encounter [Schematic–Flagellar motor diversity].

6.2.1 *Bdellovibrio bacteriovorus*

This is an average of the flagellar motors from many *Bdellovibrio bacteriovorus* cells. Working outward from the base, the major parts of the rotor are the C-ring (for Cytoplasmic), the MS-ring (for Membrane and Supramembrane), the rod, the hook, and finally the filament. They are surrounded by stationary parts: the stator ring (which is dynamic and so doesn't resolve well) and a series of bushings that allow rotation within the cell wall (the Periplasmic or P-ring) and outer membrane (Lipopolysaccharide or L-ring). Additional cytoplasmic components form the export apparatus involved in assembly (discussed on the next page).

6_2_1 Schematic: Flagellar motor diversity

As you can see in these averages of flagellar motors, different species of bacteria have evolved structural adaptations to better fit their environments. For instance, if your cell is a pathogen colonizing an animal's respiratory tract (like *Pseudomonas aeruginosa*), it will be swimming in more viscous conditions and may therefore have evolved a wider stator ring to generate more torque, along with reinforced anchors in the cell wall and outer membrane to withstand that added torque.

6.3 *Hylemonella gracilis*

Operating the flagellar motor is impressive, but so is building it in the first place. Remember that the envelope of bacterial cells is a complicated multilayered barrier. The flagellar motor has about two dozen unique components, each present in many copies, embedded in every layer of the cell envelope, with parts in the cytoplasm, periplasm (if the cell is a diderm), and extracellular space. How can your cell get all hundred or more components (tens of thousands if you count the components of the filament) where they need to go? Making the feat even more impressive, the machine builds itself, assembling from the inside out. First the components associated with the inner membrane come together, forming an "export apparatus" which pumps subsequent components across the membrane to assemble in the periplasm and outer membrane (if the cell is a diderm). The energy for this process comes from an ATPase at the base of the machine. You can see a late stage in this assembly process in this *Hylemonella gracilis* cell. The final piece to be assembled (still missing here) is the flagellar filament, which continues to assemble outward; flagellin monomers travel through the hollow tube to take their places at the tip. Flagellar motors can also disassemble, for instance when the filament is broken [More—Flagellar motor disassembly], and cells may make many new flagella throughout their lifetime.

So the flagellar motor is not just a machine for motility; it is also a machine to secrete molecules outside the cell. Bacteria and archaea contain many such "secretion systems," each specialized to transport specific macromolecules (e.g. DNA or a toxin protein) across cell envelopes – both their own and sometimes others. Secretion systems are classified by evolutionary relatedness; there are currently ~10 types recognized in bacteria, some of which are also present in archaea. The flagellar motor is an example of a Type III Secretion System. You'll see another member of this family in Chapter 9, and example of many other types in the rest of the book, starting in just a few pages.

6.3.1 *Pseudomonas aeruginosa*

When flagella break, or in some species like *Caulobacter crescentus* are ejected so the cell can attach to a surface, the motor is disassembled, usually beginning with the export apparatus. Not everything is dismantled, though; the P- and L-rings remain in place in the cell wall and outer membrane, respectively, as you can see in this *Pseudomonas aeruginosa* cell. We're not sure if there's a reason for leaving them there, but one possibility is that they may function as a plug when the hook is absent.

6.4 *Campylobacter jejuni*

Once assembled, flagella can work in different ways. The motor is bidirectional, and can rotate either clockwise or counterclockwise. Depending on the number and location of flagella on the cell (and the cell's shape), this can push the cell, pull it, or give rise to even more complicated swimming behavior. Some bacterial species, like the *Caulobacter crescentus* and *Bdellovibrio bacteriovorus* you saw at the beginning of the chapter, are monotrichous ("single haired"), with one flagellum located at one pole to push/pull the cell. Other species, like the *Campylobacter jejuni* here, have bipolar flagella—one at each end. Still others are lophotrichous ("crest-haired"), with a clump of flagella [More—Lophotrichous bacteria].

6.4.1 *Helicobacter pylori*

Many lophotrichous species, like the *Hylemonella gracilis* you saw in Chapter 3 or this *Helicobacter pylori*, have a tuft of flagella at their cell pole. In some species, though, the tuft is located elsewhere; a clump of flagella on the concave side of banana-shaped *Selenomonas artemidis* pushes cells sideways in a seesawing swimming pattern.

6.5 *Pseudomonas flexibilis*

Still other species are peritrichous ("hair around"), with multiple flagella distributed randomly around the cell, as you can see in this *Pseudomonas flexibilis*. The well-known model system *Escherichia coli* is also peritrichously flagellated. In this scheme, when the flagellar motors are all rotating one direction (counter-clockwise), the flagella form a whip-like bundle that propels the cell to "run" in a straight line. When one or more motors switch to clockwise rotation, the flagella dissociate from the bundle and "tumble" the cell to face a new direction. In the next chapter, you'll see how cells use this behavior to seek out favorable spots.

6.6 *Helicobacter hepaticus*

You may have noticed that some of the flagella you've seen in this chapter were enclosed within the outer membrane of the cell. We call these "sheathed" flagella. It's a common adaptation in pathogenic species, like this *Helicobacter hepaticus*. Flagella offer pathogens a great advantage in colonizing their hosts; hosts in turn have learned to use them to identify potential invaders. As a result, the innate immune response of many eukaryotes, from plants to insects to humans, has evolved to recognize the telltale and abundant signal of flagellin monomers in the long filament. If your cell aims to take up residence in such a host, it could therefore benefit from hiding this strongly antigenic feature.

6.7 *Borrelia burgdorferi*

If your cell is a pathogen, swimming can be very useful, but so can burrowing, for instance between cells in host tissue. How could your cell do this? Why not turn itself into a corkscrew with the equipment at hand? Some cells do just this, wrapping their flagellum around their body to back out of a tight spot, or burrow into one [More–Bacterial invasion]. Other, diderm species like the *Borrelia burgdorferi* here have turned the temporary adaptation into a permanent one: they assemble their flagella inside the cell envelope, with the filaments wrapping around between the cell wall and outer membrane. These "periplasmic" flagella are usually multiple, arising from one or both ends of the cell, and pack together, forming a helical ribbon. This helical ribbon helps give these Spirochetes ("spiral haired") their characteristic shape; mutants that can't make flagella are simple rods. Some spirochetes have additional features that may help them move around in animal hosts [More–*Treponema primitivum*]

6.7.1 *Macaca mulatta*

This unidentified bacterium has burrowed between the microvilli of a Rhesus macaque colon. You can see the sheathed flagellum wrapping around the cell body like a corkscrew.

6.7.2 *Treponema primitivum*

Treponema primitivum like this are commensal residents of the termite gut, helping break down cellulose. In addition to two periplasmic flagella, cells have arrays of bowl and hook-like structures on their surface, the function of which remains mysterious.

6.8 *Methanoregula formicica*

Archaea swim, too. And as you might expect, they use similar machinery to do so: an envelope-embedded motor that spins a long extracellular filament. Despite the structural similarity, the machinery evolved independently, another indication of the strong advantage conferred by swimming. To reflect this similar-but-not-the-same character, we call the archaeal analogue of the bacterial flagellum the archaellum. Unlike the flagellum, which is a Type III Secretion System, the archaellum is a Type II Secretion System. As you can see in this *Methanoregula formicica* cell, archaella are narrower than flagella [Schematic–Archaellum structure]. The motor is also different [Schematic–Archaellar motor structure], and uses the ATPase at the base not just for assembly, but also to power the rotation for swimming. Like flagella, archaellar motors can rotate either direction, resulting in the filaments pushing or pulling the cell.

6_8_1 Schematic: Archaellum structure

6_8_2 Schematic: Archaellar motor structure

6.9 *Thermococcus kodakaraensis*

Similar to flagella in bacteria, different archaeal species employ different numbers and patterns of archaella. Some cells have one, others have many, either distributed peritrichously (all around) as in the *Methanoregula formicica* you just saw, or lophotrichously (clumped) as in this *Thermococcus kodakaraensis*. In *T. kodakaraensis* and related species, an additional structure—a large conical plate—is seen in the cytoplasm, perhaps providing leverage for the multiple motors. The plate has a unique structure [More—Cone structure] and may act as an organizing center akin to the polar PopZ structure we discussed in the last chapter [More—Organizing center].

6.9.1 *Thermococcus kodakaraensis*

The cone doesn't come to a point at the tip, but rather is a conical frustum (open at the top), resembling a lampshade. In the center of the tip is a small ring, as you can see in top view in this lysed, flattened *Thermococcus kodakaraensis* cell. We don't yet know the function of this ring; perhaps it helps nucleate the rest of the structure?

6.9.2 *Thermococcus kodakaraensis*

As you can see more clearly in this partially-lysed, flattened *Thermococcus kodakaraensis* cell, the conical structure in the cytoplasm is attached to more than just the archaella. It is also associated with

chemosensory arrays (discussed in the next chapter) and DNA, as you can see from the ribosome-excluding zone. This structure may therefore perform an analogous function to bacterial organizing proteins such as PopZ, tethering cellular components into a de facto pole for, in this case, a round cell.

6.10 *Myxococcus xanthus*

If your cell lives on a surface, what's the best way to get around? How about using a grappling hook? Some bacteria, like this *Myxococcus xanthus*, use a Type II Secretion System related to the archaeal motor to pull themselves around their environment. As you can see, the structure looks familiar: a motor embedded in the envelope with a long extracellular filament. In this case the filament is called a pilus ("hair" in Latin). Bacteria and archaea make many kinds of pili (also generically called fimbriae ("fringe")) and you'll see some of their other functions in later chapters. The *M. xanthus* pili, classified as Type IV pili, don't function as propellers like flagella or archaella, but rather extend, attach to a surface, then retract to pull the cell toward the attachment point [Schematic—Type IV pilus structure]. The pilus motors are the strongest known in nature, and can retract pili at up to 1 m/s; the combined action of multiple pili leads to extremely rapid "twitching" motility over the surface. The motor structure, or basal body, remains intact even when no pilus is assembled. These rod-shaped cells have many basal bodies at both cell poles; to switch direction, the cell simply disassembles the pili on one end and builds new pili from the machines on the other.

In addition to attaching to a surface, the pili can also attach to another *M. xanthus* cell. This enables the cells to move over surfaces en masse. Combined with their predatory practice of eating other bacteria, this property has led them to be compared to packs of wolves hunting down their prey.

6_10_1 Schematic: Type IV pilus structure

A series of rings anchor the basal body in the cell envelope. This rigid structure provides leverage for an ATPase at the base to rotate an adaptor in the inner membrane. We think that when it spins in one direction, the adaptor scoops pilin monomers diffusing in the inner membrane into the assembling pilus. Once the pilus has reached its target, attachment is sensed by the basal body (we still don't know how). As a result, the assembly ATPase dissociates and a second, homologous ATPase takes its place. This disassembly ATPase spins the adaptor in the opposite direction, escorting pilin monomers back into the inner membrane, ready to join another growing pilus.

6.11 *Flavobacterium johnsoniae*

Other bacteria use different machinery to move over surfaces. *Flavobacterium johnsoniae* like this cell use a Type IX Secretion System to secrete adhesive filaments. These filaments move on a helical track around the cell, and thereby the cell moves forward on a surface. The power for this gliding movement is thought to come from rotary motors anchored in the cell wall that propel the adhesins along their track in a rack-and-pinion fashion, but we still don't understand the mechanism in detail.

6.12 *Mycoplasma pneumoniae*

Another, familiar, way to get across a surface is to walk. For this to work, though, you need to be able to change the conformation of your body. This is possible if your cell lacks a cell wall or surface layer, like this *Mycoplasma pneumoniae*. Like the *Mycoplasma genitalium* you saw in Chapter 2, these cells are intracellular pathogens, so they don't need to buttress their membrane against differences in osmolarity. As a result, they are conformationally flexible. This may allow them to use a leg-like internal structure called a terminal organelle [Schematic—Terminal organelle structure] to crawl, or more elegantly “glide,” across a surface. The exact mechanism is still unclear, but one possibility is that a hinge-like conformational change in the terminal organelle extends and contracts the back of the cell with respect to the front, similar to the movement of an inchworm (but less exaggerated). Combined with adhesion proteins on the cell surface, this might propel the cell forward.

The skeleton-like terminal organelle gives these cells their characteristic flask shape. In combination with their minimal cell envelope, it can also give rise to an unusual method of cell division. In some species (or mutants) that lack the division protein FtsZ, *Mycoplasma* cells still manage to divide. They replicate their terminal organelle normally as you can see this cell has done and then the two copies simply walk away from each other, stretching the mother cell between them until the membrane spontaneously separates to produce two daughters.

Keep in mind that these are simply some of the ways we already know bacteria and archaea get around, and we continue to discover new ones.

6_12_1 Schematic: Terminal organelle structure

7

Navigation

Navigation

8

Lifecycle

Lifecycle

9

Interaction

Interaction

10

Viruses

Viruses

11

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