

7.11 Biomechanics of Cell Motility

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7.11.1	Introduction	168
7.11.2	Cellular Movements in Fluid	169
7.11.2.1	Prokaryotic Swimming	170
7.11.2.2	Eukaryotic Swimming	173
7.11.2.3	Modeling the Biomechanics of Swimming	177
7.11.3	Gliding and Crawling on a Surface	180
7.11.3.1	Gliding Motility of Bacteria	180
7.11.3.2	Eukaryotic Cell Crawling	182
7.11.3.3	Modeling Eukaryotic Cell Crawling	186
7.11.4	Conclusions	187
	Acknowledgments	188
	References	188

Abbreviations

CCW	counterclockwise
CW	clockwise
IBM	immersed boundary method
JPC	junctional pore complex
MSP	major sperm protein

PilB	nucleotide binding protein
PilC	polytopic inner membrane protein
PilD	prepilin peptidase and methylase
PilQ	outer membrane protein
RFT	resistive force theory
SBT	slender-body theory

7.11.1 Introduction

Imagine this: You are placed within a capsule. In the walls are numerous small shutters. If you open a shutter, you can peer out and gain an extremely limited view of the world outside. It is also possible to stick small objects out through some of the openings or, if something passes near enough, you might be able to pull it in. The inside of the capsule is comfortable. There are numerous tools around and plenty of supplies. Food is scarce, though, and after the first few uneventful hours, you start to get hungry. You look out of the openings but don't see any food in the immediate vicinity. What do you do?

This scenario, though contrived from the perspective of a large multicellular organism, may represent one of the first truly important questions that our primordial, single-celled ancestors faced. The compartment described here is just that, a single cell, with the capsule as the outer surface of the cell and delineating the inside from the outside. The shutters represent any input or export organelle in the cell surface, such as ion channels, pumps, receptors, or secretion apparatus. The tools and supplies are the proteins, phospholipids, and nucleic acids. In order to grow and divide, though, the cell needs nutrients from the environment. Therefore, if there is no food nearby, then the cell needs to move. (There are, of course, other reasons why a cell would need to move. For example, during the development of an organism, cells move to position themselves in the correct place, and white blood cells move to track down pathogens in our blood supply.) But how can a single-celled organism do that? In general, there are probably an uncountable number of ways; however, there are a few factors that greatly limit the possibilities.

In order to move, cells exert force on the environment and, for cellular-sized objects, resistive drag forces are comparable to the forces that a cell can produce. Therefore, inertia is negligible: A cell that stops producing force is almost immediately brought to a halt by the environment. The effective absence of inertia means that the net force on a motile cell is zero. Here there is a subtlety. If a cell exerts force on the environment, then the environment must push back with an equal but opposite force. This is the thrust force that propels the cell. But the environment also resists the motion of the cell and exerts a force on the cell that opposes the motion. It is the environment that allows motion and yet impedes it.

There is yet another sticky part: To exert force on the environment, a cell needs to grip its surroundings. Being able to adhere to the environment is good for this and, typically, the better a cell's adherence is, the more force it can exert against its substrate. To move through the environment, though, a cell needs to slip with respect to the surroundings. Being well adhered is bad for this. The more the cell is adhered, the harder it is for the cell to go anywhere. To further complicate things, if the cell wants to be able to move over long distances, then whatever mechanism it uses to generate force against the environment should be cyclical. That way, a cell can move an arbitrarily far distance, just by repeating the cycle as many times as needed.

To put these issues into perspective, let's consider a human walking. Except rather than normal walking, let's consider walking through waist-deep tar. To move forward, you would need to exert force on the floor with your feet. You would push backwards in order to move forwards. The ability to exert this

force is only possible because of the friction between your feet and the floor. Without friction, your feet would just slip along the surface and you wouldn't go anywhere, as most people know if they have tried to walk on ice. At the other extreme, if the floor were too sticky, then locomotion would be equally difficult. A prime example is that of the unfortunate fly that happens to land on fly-paper. The tar surrounding the lower half of your body, though, would resist your movement and could balance the force that the floor exerts back on you. Therefore, to move at a constant velocity your feet would exert a net force on the floor, the floor would exert a force back on your feet, the tar would exert a force on you preventing your motion, and you would exert a force back on the tar. All told, the net force on you would be zero, as would the net force that you exert on the environment. To create a cycle, you would merely alternate which foot is in contact with the ground and swing the other foot forward.

From these considerations, it is clear that the environment will play a crucial role in determining what motility strategies are possible. A cell that is fully immersed in a fluid will not be able to use the same technique to move as a cell that is in contact with a surface. Another important consideration for a cell is how it is going to generate force against the environment. In most cases, force is generated by moving parts. Thinking back to the scenario presented at the beginning of this chapter, our cell was defined by a capsule that separated the inside of the cell from the outside. For a eukaryotic cell, this capsule represents the cell membrane and possibly the actin cortex, which is a meshwork of filaments that lies next to and is attached to the cell membrane. In a bacterium, the capsule represents the inner membrane and cell wall, and also the outer membrane in Gram-negative bacteria. If this 'capsule' is pliable, then it is possible for the capsule itself to be the moving part that exerts force on the surroundings. However, in other cases, the capsule is fairly rigid. For example, the cell wall in many bacteria is relatively stiff, which helps protect the bacterium from harsh environments but, as we will find out, will require the bacterium to stick things outside of it in order to move.

The goal of this chapter is to describe some of the many ways that cells have developed to move. The focus will be on the biomechanical aspects of single cell motility, which means that we will explore the mechanisms that cells use to produce force against their environments, and how the environment responds, thereby leading to a moving cell. In general, cells interact with two types of environments: A cell can be immersed in a fluid or in contact with a substrate. Therefore, the primary subdivision of this chapter lies with environment. The first half of the chapter will look at cellular motility in a bulk fluid, which, as with multicellular organisms, is generically known as swimming. Then the second half of the chapter will explore the mechanisms that cells have devised to move along more rigid substrates. In order to avoid any major taxonomic bias, both prokaryotic and eukaryotic cells will be covered. We will find that the biomechanical considerations involved in motility are strongly dependent on environment, and less so on cell type. In addition to just describing the types of motility, this chapter also aims to develop a basic understanding of the physics involved in cellular motility. As such, each section is accompanied by an overview of the

mathematical analysis of the biomechanics of these types of motions.

7.11.2 Cellular Movements in Fluid

In this section, the focus is on how cells move when they are completely submerged in a fluid. But before we dive in, the first point that needs to be addressed is the difference between active motions and passive motions.

Cells are small. Though they are much larger than the size of the fluid molecules around them, collisions between the fluid molecules and the cell can still be important. In regard to the movement of a whole cell, these molecular collisions lead to random displacement of the center of mass over time, a process known as Brownian motion. This Brownian displacement of the center of mass requires no effort from the cell; it is driven completely by the collisions that come from the external fluid molecules, and is therefore a passive form of motility. Another form of passive motility is advection, where a cell gets carried along by the flow of the fluid that it is submerged in. Like a log floating down a river, the fluid does the work, not the cell. On the other hand, if the cell has to expend energy to do something in order to move, then it is an active form of motility. This chapter will focus on active motility. We will consider what types of processes a cell can use to move that aren't reliant on diffusion or advection. That is, if a cell wants to determine its own fate, rather than relying on the benevolence of Nature, what can it do?

The first question one should ask, though, is: Is it worthwhile to expend energy to move? To push an object through a fluid at constant velocity requires a force. This force, which is commonly referred to as a thrust force, is balanced by the resistive drag force that the fluid exerts back on the object. (In agreement with Newton's second law, the sum of the forces on the object is zero, as is the acceleration). For cellular-sized objects, the drag force is proportional to the size (length) and velocity of the object and the viscosity of the fluid.¹ A cell must expend energy to produce the thrust force. If, instead, the cell does nothing, the cell will still move about randomly due to Brownian motion. Which is better for the livelihood of the cell? To answer this question, consider a cell that is in an area where there is no food. We expect that the farther the cell can travel before using up its stored energy, the more likely the cell is to find food and survive. The cell has a baseline metabolism corresponding to the rate at which it burns through its stored energy when it is sitting still. On average, Brownian motion moves the cell a distance that is proportional to the square root of time and is inversely proportional to the square root of the size of the cell.² A motile cell uses up its stored energy faster, at a rate proportional to the square of the velocity (the power expended by the cell is the force times the velocity), but the cell can move more rapidly when it is actively swimming. Consideration of these two scenarios suggests that it is more effective for small cells (i.e., cells smaller than a micron in length and width) to be nonmotile, whereas it is beneficial for cells that are larger than this size to be motile, at least if food is scarce.³ Since most bacteria and all eukaryotic cells are larger than a micron, many cells have figured out how to actively move.

7.11.2.1 Prokaryotic Swimming

Well over 2 billion years ago, before the archaeobacteria and eubacteria diverged, some cells had figured out how to move through a fluid.^{4,5} For those unfamiliar with these primitive cells, the basic structure of the cell is quite simple. Neither type of cell has a nucleus, so the inside of the cell is a single compartment, known as the cytoplasm, which includes water, ions, proteins, and DNA (Figure 1(a)). A bilayer membrane composed of lipid molecules separates the inside of the cell from the outside of the cell. By regulating the flux of charged molecules across the membrane, the cell is able to maintain an electrostatic potential across the membrane. In bacteria, this membrane is known as the inner membrane or cytoplasmic membrane. Just beyond the membrane, there is a cell wall that is constructed from a meshwork of crosslinked filaments. The exact biochemistry of the cell wall differs between the bacteria and the archaeobacteria, but in both cases the wall provides a protective layer for the cell and is also required to maintain the cell shape. In regard to shape, these cells come in a variety of shapes and sizes. The most common shapes are spherical (also known as coccoid), cylindrical (also known as rod-shaped or bacillus), bent rod shape, helical, and branched, and cells range in size from submicron up to tens of microns long. The most commonly studied swimming bacteria are rod shaped, of the order of a micron in diameter, and a few microns long. For example, *Escherichia coli* and *Salmonella* are roughly 1 μm in diameter and 2–5 μm long, and *Bacillus subtilis* is around 0.7 μm in diameter and about 4 μm long.⁶

Since the cell wall provides structural rigidity to the cell, it is often quite stiff. In addition, many bacteria are quite small. Since it is harder to bend a small object than it is to bend a longer object that is made out of the same material, many bacteria cannot bend and flex in order to move through a fluid. Therefore, the most common form of swimming for bacteria and archaeobacteria involves long, thin helical filaments, known as flagella or flagellar filaments, which extend out of the cell into the surrounding fluid (see Figure 1). Bacteria can have anywhere from one flagellum (e.g., the swarmer cell of *Caulobacter crescentus*⁷) up to hundreds (e.g.,

*Spirillum volutans*⁸), with the most commonly studied bacterial swimmers (*E. coli*, *Salmonella*, and *B. subtilis*) having between four and ten flagella per cell.^{9–11} These flagella are attached via a hook filament to a molecular rotary motor that is anchored into the inner membrane of the cell. Proton or sodium ion flux through the motor causes the motor to rotate, which turns the flagellum in the fluid.¹² Rotation of the helical flagellum pushes on the fluid, and the commensurate reaction force provides the thrust force that propels the bacterium. In what follows, a brief description is given of what is known about the flagellar motor and the flagellar filament, and how bacteria use these structures to get where they need to go. Then some alternative mechanisms that some bacteria use to swim will be described.

The flagellar motor is a nanomachine that converts electrical current (the flow of ions) into mechanical work and also serves as a transport apparatus that exports the proteins that build the hook and flagellum (see Figure 2). At top speed, the motor rotates in excess of 100 Hz. The motor, which can also be called the basal body, is a complex macromolecular structure that is composed of ~ 20 proteins in *E. coli*.^{9,12} As with any rotary motor, the basic design of the flagellar motor can be broken down into two main parts, a stator and a rotor. As their names imply, the stator remains fixed in position when the motor is on, and the rotor rotates. The flagellar motor can actually rotate in either direction, clockwise (CW) or counterclockwise (CCW), with the direction of rotation controlled by a chemosensory switching mechanism. When one builds an engine or a motor, one of the important properties of that engine is the power output. Since power can be computed as the force times velocity, or torque times angular velocity for a rotary motor, a rotary motor is often characterized by its torque–speed relationship, which describes how fast the motor turns when a counter-torque is applied externally to the motor. The torque–speed relationships for the flagellar motors of *E. coli*, *C. crescentus*, and *Vibrio alginolitycus* have been measured.^{13–15} For these motors, the torque supplied by the motor is observed to be roughly constant at low speed and then decreases linearly above a certain rotational rate.

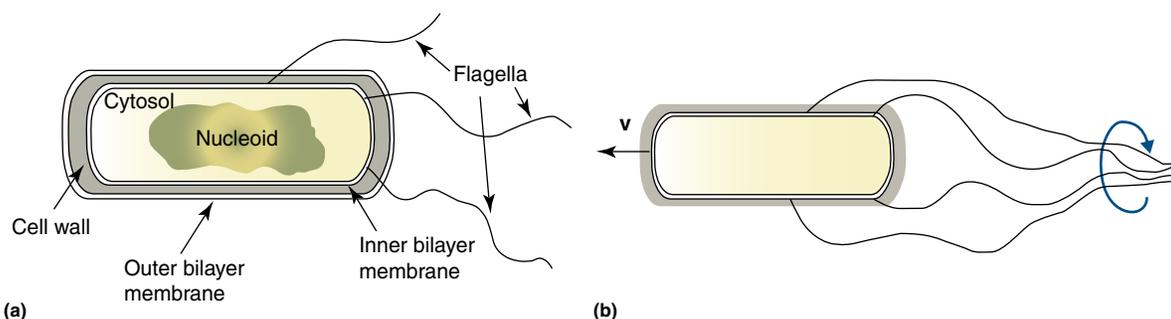


Figure 1 Schematic of a bacterium. (a) A typical Gram-negative bacterium. The inside of the cell is a single compartment that contains the nucleoid (DNA) and the cytoplasm. The inner membrane separates the inside of the cell from the outside. The structural rigidity of the cell comes from the cell wall, which lies just outside the inner membrane and is composed primarily of a meshwork of glycan strands connected by peptide bond crosslinks. The outer membrane of the cell encloses all of these structures (Gram-positive bacteria do not have this outer membrane). Most swimming bacteria extrude long helical filaments, known as flagella, out into the fluid. (b) Rotation of the flagella by the flagellar motor causes the flagella to bundle together. This rotating flagellar bundle pumps fluid away from the cell, which propels the cell in the opposite direction with speed v .

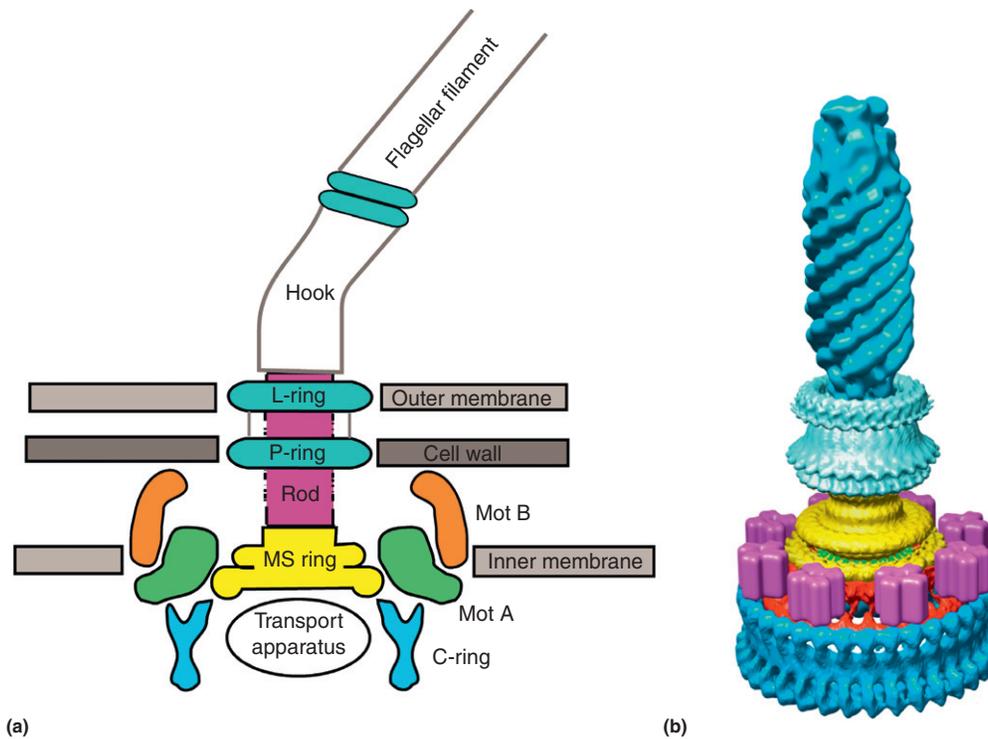


Figure 2 (a) Schematic of the bacterial flagellar motor structure, showing the C, MS, P, and L rings, the Mot A/B complex, the transport apparatus, the rod, the cell wall, and the cell membranes. The C ring and the Mot A/B complex are believed to function together as the stator for the motor, and the MS ring is the rotor. The hook and flagellar filament attach to the rod, and are outside the outer membrane of the cell. (b) An image of the flagellar motor that was generated from EM data. A portion of the hook is shown attached to the motor. Image courtesy of D. J. DeRosier.

Based on electron micrographs, the motor is subdivided into four ring-like structures, known as the C, MS, P, and L rings.⁹ In addition to these rings, a central rod conveys the torque from the motor to the hook and flagellar filament. This central rod is composed of four proteins: FlgB, FlgC, FlgF, FlgG. The C ring is the cytoplasmic domain of the motor and is composed of two proteins, FliM and FliN. These proteins, along with FliG, which is the cytoplasmic part of the MS ring, are involved in controlling the rotational direction of the motor.^{16,17} The C ring surrounds a knob-like structure that is constructed from eight other proteins (FlhA, FlhB, FliH, FliI, FliO, FliP, FliQ, and FliR) and is believed to be the transport apparatus associated with the motor.^{18–24} The MS ring is composed of a single protein, FliF. The combination of the C and MS rings is commonly thought to function together as the rotor.⁹ The other two rings, P and L, presumably act as bushings that allow the passage of the rod through the peptidoglycan and outer membrane respectively.

So where is the stator? Two additional proteins, MotA and MotB, which are not readily observed in the electron micrographs of the purified motor, form the stator.²⁵ Both of these proteins span the cytoplasmic membrane; however, MotB is located predominantly in the periplasmic space.^{26,27} MotB has a peptidoglycan binding domain²⁸ and therefore is believed to anchor the stator to the cell wall material, which allows the stator to remain stationary with respect to the bacterial cell body when torque is applied to the rotor portion of the motor.

But how does this conglomeration of proteins conspire to produce a functioning rotary motor? In other words, what is the interaction between the MotA/MotB complex (stator) and the C/MS rings (rotor) that leads to rotation and torque generation when ions flow through the motor? Since the MotA/MotB complex spans the inner membrane of the cell, protons outside of the cell could be driven through the complex by the membrane potential. The effective potential that protons feel also depends on the pH gradient across the membrane. In *E. coli*, grown at pH 7, the membrane potential is -120 mV,⁹ and the maximum torque that the flagellar motor can exert has been estimated to be around 4600 pN nm.²⁹ From these numbers it is possible to estimate the number of protons that flow through the motor per revolution. The membrane potential does 0.12 eV worth of work on each proton that passes through the motor. If all of this energy is available to produce torque, then we estimate that it requires at least 381 protons per revolution, which is roughly comparable to the measured value of 1200.³⁰ But how does ion flow lead to molecular rotation? There are currently thought to be four MotA and two MotB proteins that comprise the stator.¹² It has been suggested^{31,32} that proton binding to a residue on the MotB protein leads to a conformational change of the stator. This conformational change of the stator then pushes on FliG in the rotor through either steric or electrostatic interactions, which leads to the rotation of the rotor.^{31,32} It is also suggested that the position of the rotor acts to either allow or forbid transport of ions through the ion channel.

Mathematical modeling of the flagellar motor has shown that this picture is consistent with the torque–speed relationship of the flagellar motor.³³

The torque from the flagellar motor is transmitted to a long helical filament (flagellum) via a short filament that is roughly 50 nm in length, known as the hook.³⁴ The hook is composed of a single protein, FlgE,³⁵ and in *E. coli* and *Salmonella* the flagellum is also composed of a single protein, FlgC (also known as flagellin).⁹ In other bacteria, the flagellum can be constructed from multiple proteins and the core filament is sometimes encased by a sheath. Here we will consider the flagellar filament of *E. coli* and *Salmonella*, for simplicity. Both the hook and the flagellum are comprised of 11 parallel rows of subunits (known as protofilaments) that form a hollow tube structure with a diameter of approximately 20 nm.³⁴ The hook is very flexible to bending, with a bending persistence length (the length over which thermal fluctuations can bend the filament) estimated to be between 40 and 120 nm,³⁶ which allows the hook to act like a universal joint (i.e., it conveys the torque from flagellar motor to the flagellum, but allows the flagellum to rotate fairly freely with respect to the bacterial cell body). (The persistence length of a filament is related to Young's modulus of the material. For a solid filament with cross-sectional radius a , the persistence length is roughly equal to $Ea^4/k_B T$, where E is Young's modulus and $k_B T$ is thermal energy.) The flagellum is significantly stiffer, with a persistence length estimated to be around 1 mm.^{37–41} The flagellum can take on many different stable shapes, which is a property known as polymorphism. Of these possible shapes, there are two straight states and multiple possible helical states. In *E. coli*, when the bacterium is smoothly swimming and the flagella are rotating CCW, the flagellum is in what is known as the normal state, which is a left-handed helix with helix radius of 0.2 μm and pitch of 2.3 μm .⁴² When the flagellar motor switches directions the flagellum can flip into one of the other stable states.^{42,43} Steady fluid flow past the flagellum can cyclically convert the flagellum from a right-handed helix to a left-handed helix.⁴⁴ The ability of the flagellum to have multiple stable configurations is due to the flagellin monomer, which can have two stable conformations (i.e., it is bistable).⁴⁵ A model that considers the bistability of the flagellin monomers is able to predict 12 flagellar configurations, two straight and ten helical states.^{46,47} A more careful mathematical analysis suggests that there may actually be a continuum of possible states.⁴⁸

A problem for most bacteria is that they are small; therefore, it is impossible for them to distinguish whether there is more food at their head or tail. So how do all these pieces work together to get an *E. coli* where it wants to be? As previously mentioned, an *E. coli* cell has four flagella per cell, on average. If all the flagellar motors are rotating CCW, the flagellar filaments rotate in the external fluid and wrap around each other into a tight bundle. In this tight bundle, the flagella effectively act like a single rotating helix, which acts to pump the fluid away from the cell and pushes the bacterium in the opposite direction (**Figure 1(b)**). During this phase, which is known as a run, cells swim at around 30 $\mu\text{m s}^{-1}$.⁹ Periodically, one or more motors reverse direction, and the corresponding filaments come out of the bundle, which causes the cell to erratically reorient, or tumble.⁴³ In the absence of external

cues, the bacteria tumble every second or two. The bundling and unbundling of the flagella are driven by hydrodynamic interactions.^{49,50} During tumbles, the CW-rotating flagella transition from the normal helical state, first to a right-handed 'semi-coiled' state and then to the 'curly-1' helical form.⁴³ When the motor reverts to CCW rotation, the flagellum flips back to the normal state. The progression of runs and tumbles leads to a random walk-type movement that allows the bacterium to randomly sample its environment in search of nutrient.² This motion leads to diffusive behavior of a population of bacteria, with a diffusion coefficient $D = v^2 \tau / 3$, where v is the bacterial swimming velocity and τ is the average time between tumbles. A signaling pathway inside the cell responds to changes in external nutrient and alters the frequency that the flagellar motors reverse direction accordingly.^{51,52} When the bacterium is moving in a direction of increasing nutrient, the cell reverses less frequently. In this fashion, the bacterium executes a biased random walk that moves the bacterium in the direction of increasing nutrient. This type of random search for nutrient is known as run-and-tumble chemotaxis.

Though run-and-tumble chemotaxis is quite common amongst bacteria, some bacteria with flagella have devised other means of getting where they want to be. For example, *Rhodobacter sphaeroides* can only rotate its flagella in one direction.⁵³ These cells change their swimming direction by either stopping or changing the speed of the flagellar rotation. Many marine bacteria use a strategy known as run and reverse, where rather than randomizing its direction in the forward sense, the bacterium swims backward, roughly along its previous path.⁵⁴ And some sulfide-oxidizing marine bacteria may be large enough to sense a spatial gradient.⁵⁵ These bacteria have flagellar bundles on both sides of the cell and are able to make U-turns during swimming in order to get back to favorable oxygen concentrations.

Spirochete bacteria have come up with another way to use the flagellar system to drive their motility. Spirochetes are long, thin bacteria, with cell diameters in some species being smaller than 0.2 μm and lengths that can exceed 20 μm .^{56,57} Since these cells are long and thin, it requires less force to bend the cell wall. Therefore, rather than extruding their flagella out into the external fluid, they encase their flagella in between the cell wall and the outer membrane (this region is known as the periplasmic space). The flagella are attached to flagellar motors that are structurally similar to the motors found in other bacteria.^{58–60} The motors are located subterminally at both ends of the bacterium, and the flagella extend in toward the center of the cell. Depending on species, there can be one to hundreds of flagella attached at each end.⁵⁷ In some species the flagella are short and only extend in a few microns from the end of the cell, while in other species the flagella are long enough to overlap in the center. This placement of the flagella allows them to interact directly with the cell wall and, in some species, the flagella are known to serve a skeletal function, in addition to their involvement in motility. For example, *Borrelia burgdorferi*, the bacterium that causes Lyme disease, has a periodically undulating, nearly planar shape (known as a flat-wave shape) (**Figure 3(a)**). Mutants lacking flagella, however, are rod shaped.⁶¹ Mathematical modeling has shown that coupling of helical filaments to an elastic cylinder will naturally lead to this flat-wave

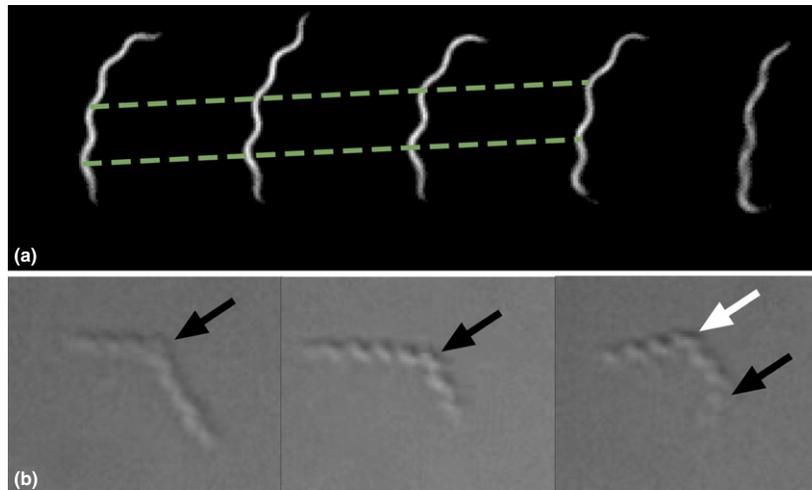


Figure 3 (a) *Borrelia burgdorferi* swimming in gelatin. The time series shows the traveling wave deformations that drive the swimming of *B. burgdorferi*. The green dashed lines highlight the rearward motion of the traveling wave as the bacterium swims. Images courtesy of S. Dunham-Ems and J. D. Radolf. (b) *Spiroplasma* swims using traveling kinks. In the first panel, a kink (black arrow) exists where the cell body flips from one handedness (e.g., a right-handed helix) to the opposite handedness. This kink moves rearward. In the final frame, a new kink is now apparent (white arrow) where the cell body flips back to its original handedness. Images courtesy of J. Shaevitz.

shape.³⁸ Rotation of the flagella in the periplasmic space produces traveling wave deformations of the cell body, and this wave-like motion propels the bacterium through the external fluid at speeds of around $5 \mu\text{m s}^{-1}$ (Figure 3(a)).⁶² The biomechanics of the interaction of rotating, flexible flagella in contact with a flexible cell body are quite complicated, yet some progress has been made in explaining the complex shape changes that accompany the motility of the Leptospiraceae.⁶³ Some species of spirochetes can cause disease. What role does this motility mechanism play in the ability to infect hosts? Spirochetes are known to be able to swim faster in gel-like media, which may explain their ability to navigate through tissue.^{62,64–67} In addition, the mammalian immune system can recognize flagellin.^{68,69} Therefore, positioning the flagella inside the outer membrane may help cloak the bacterium from innate immune response.

Not all swimming bacteria use flagella to swim. Some bacteria have devised other means for moving through fluids. *Spiroplasma*, for example, is a helical-shaped bacterium that is between 4 and 10 μm long and 0.4 μm in diameter. One species of *Spiroplasma* causes corn stunt disease, which is one of the most economically important diseases of maize in North, Central, and South America.^{70,71} Unlike most bacteria, *Spiroplasma* does not have a cell wall. The cell shape is maintained by a number of filaments that span the length of the cell and are anchored to the cell membrane.^{72,73} *Spiroplasma* swim at speeds of around $3 \mu\text{m s}^{-1}$ by propagating a pair of kinks along the body axis of the cell, with the bacterium propelled forward as the kinks move rearward^{74,75} (Figure 3(b)). Kinematic analysis of *Spiroplasma* swimming suggests that the shape may have evolved to optimize this mechanism.^{76–78} *Synechococcus*, a marine species of cyanobacteria, is an ellipsoidal-shaped cell (0.7–0.9 μm in diameter and 1–2.5 μm in length) that zips through water at speeds of $5\text{--}25 \mu\text{m s}^{-1}$.^{79–81} These cells do not have flagella, and the mechanism by which they swim is still unknown. It has been

suggested that *Synechococcus* might produce small-amplitude traveling-wave undulations of the surface in order to swim,⁸² but this remains a Sherlock Holmesian speculation (i.e., when all other options have been exhausted, what remains must be the truth; however, it is far from clear that all other mechanisms have been considered). Even more perplexing is a newly isolated halophilic (i.e., they thrive in an environment with very high concentrations of salt), wall-less bacterium from the Red Sea. These bacteria have a central coccoid body with one or two ‘tentacle-like’ projections.⁸³ These tentacles alternate between straight and helical, and cell motility may occur by repeated contraction and extension of these appendages. The biomechanical mechanisms that underlie this extension and coiling are completely unknown and highly intriguing. These are probably but a sampling of the many ways that bacteria have figured out to swim. Indeed, it is currently estimated that only 1–10% of the bacterial species in nature have been grown in the laboratory.⁸⁴ It is therefore reasonable to expect that many more novel swimming mechanisms will be discovered as more species of bacteria are unearthed.

7.11.2.2 Eukaryotic Swimming

Like bacteria and archaeobacteria, many eukaryotic cells can swim. And, like most swimming bacteria and archaeobacteria, swimming eukaryotic cells use thin appendages in order to generate the thrust necessary for swimming. However, where bacteria use a fairly rigid flagellar filament that is rotated by a motor attached at one end, the thin extensions of the eukaryotes (known as cilia and flagella) actively bend and twist during swimming, driven by an array of molecular motors distributed along the length of the appendage.

Cilia and flagella are very similar, with the primary difference between them being their length and the number per cell. A flagellated eukaryotic cell has one or two long flagella, where

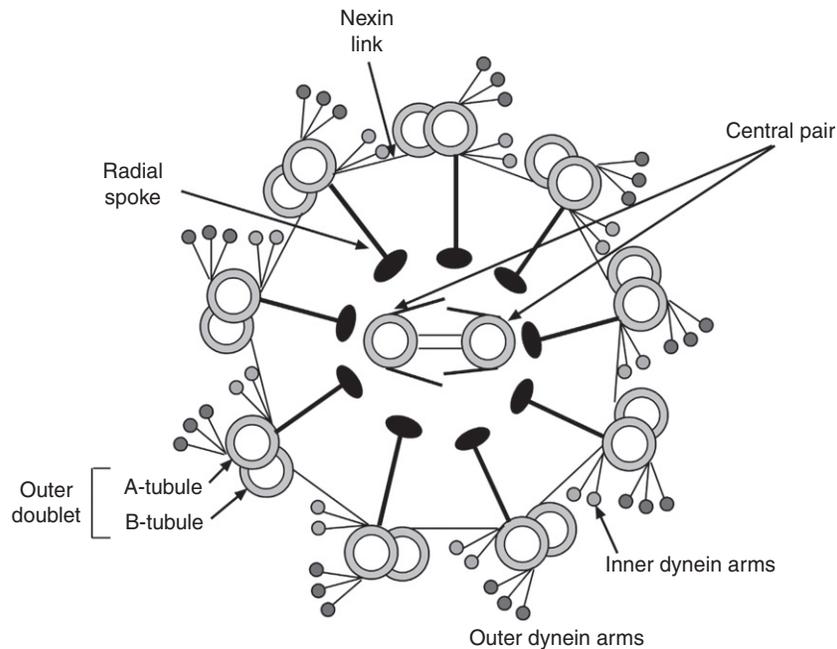


Figure 4 Schematic of the structure of the axoneme, shown in cross section. See text for details.

a ciliated cell typically has a number of short cilia. It is important to note that even though bacteria and eukaryotes can swim using a long thin appendage known as a flagellum, these organelles are very different. The main component of cilia and flagella is an active structure known as the axoneme (**Figure 4**). The axoneme is composed of a bundle of microtubules, arranged in what is known as a '9 + 2' configuration.⁸⁵ A microtubule is one of the cytoskeletal components of eukaryotic cells, which provide structural rigidity to cells and also are involved in intracellular transport. The structure of a microtubule is similar to that of the bacterial flagellum; it is built from a single protein known as tubulin, with the tubulin monomers arranged in a hollow tube-like structure.⁸⁶ In the '9 + 2' configuration of the axoneme, there are nine microtubule doublets that form yet another hollow tube-like structure. These microtubule doublets are a pair of microtubules that are fused together along their length.⁸⁵ One of the pair of microtubules (known as the A-tubule) is a complete microtubule composed of 13 protofilaments of tubulin. The other microtubule only has 10–11 protofilaments and is known as the B-tubule. These nine doublets are aligned in a ring with their long axes roughly parallel to the centerline of the axoneme. Down the center of the tube structure sit two singlet microtubules that are linked at intervals along their length, known as the central pair.⁸⁵ Three separate structures are observed to repeat at intervals along the length of the axoneme: a pair of dynein arms (spaced on average every 24 nm⁸⁵), nexin links (at ~96-nm intervals⁸⁷), and radial spokes. The dynein arms project off of each A-tubule. The inner arm connects to a band of proteins known as the nexin links, and this combination of dynein arms plus nexin links hold the doublets in their circular arrangement.⁸⁸ The second dynein arm that projects off the A-tubule is known as the outer dynein arm. Both of these dynein arms are composed of one to three dyneins, which are motor proteins that can exert force

on microtubules. Radial spokes project inward from the microtubule doublets and terminate in a globular head close to the central pair.⁸⁵ In addition to these structures, there are two sets of arms that project off the central pair of microtubules, forming a sheath about it.

Cilia and flagella produce thrust using traveling bending and twisting waves that propagate down the length of the axoneme. Therefore, at a very phenomenological level, swimming is similar to that of the spirochetes (see previous section). How does the axonemal structure lead to these traveling waves? Dynein motors use energy gained from ATP hydrolysis to exert forces on microtubules. These forces are unidirectional and are predominantly directed along the length of the microtubule. Therefore, if we consider a simple system of two microtubules that are connected by dynein motors, activity of the dynein motors will cause the microtubules to slide with respect to one another. In the axoneme, the same thing happens: The dynein arms connected to the A-tubule interact with the B-tubule on a neighboring microtubule doublet and cause the two doublets to slide with respect to each other.^{89,90} Because the doublets are linked mechanically, sliding of the microtubules is converted into bending of the axoneme. Indeed, it is possible to remove the mechanical linkage between the doublets in the axoneme using trypsin. Under these conditions, the microtubule doublets slide apart from one another in the presence of ATP.^{91,92}

An issue here is that the axoneme contains a large number of dynein motors that must coordinate their activity in order to produce the traveling wave deformation that drives motility (see **Box 1**). To create a bend in the flagellum, motors on one side of the axoneme should be engaged, and the motors on the opposite side should not. To create a bending wave along the length of the flagellum, the activity of the motors must switch sides periodically along the length, and to create traveling waves the active sides must switch periodically in time.

Box 1 How many dyneins does it take to bend a eukaryotic flagellum?

Axonemes are quite stiff. Each microtubule has a persistence length of 4–8 μm ,^{93,94} and the persistence length of a rat sperm flagellum has been measured to be around 5 μm in sea urchin sperm and around 100 μm in rat sperm!^{95,96} This means that to bend a sea urchin flagellum into a circle with a 5- μm radius of curvature (which is a typical radius of curvature⁹⁷) requires a torque of 4×10^3 pN μm . A single dynein molecule can produce a force of 6–7 pN.⁹⁸ To estimate the number of dyneins required to bend the flagellum, we estimate the torque from a dynein motor by multiplying this force by the radius of the axoneme, 100 nm. Therefore, to produce the bends in the axoneme that produce thrust requires around 10^3 dyneins per micron of length, which agrees well with the actual number.

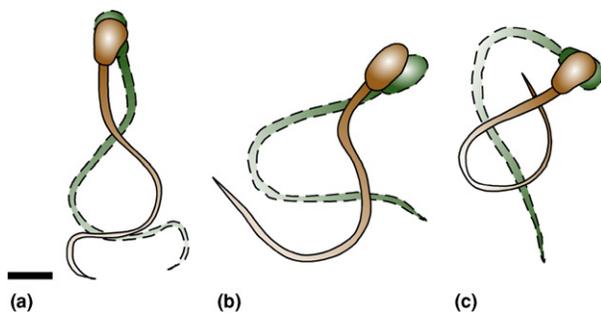


Figure 5 The deformations of a bull sperm flagellum. (a) A schematic of an activated sperm showing the symmetrical flagellar beat pattern. (b), (c) Hyperactivated sperm exhibiting high-amplitude, nonsymmetrical beating. Each drawing represents two instances during sperm swimming that are 1/60 second apart. Scale bar = 10 μm . This figure is a redrawing of Figure 1 from Marquez, B.; Suarez, S. S. Different signalling pathways in bovine sperm regulate capacitation and hyperactivation. *Biol. Reprod.* **2004**, *70*, 1626–1633. Copyright by Biology of Reproduction.

Mathematical and computational modeling have shown that this coordination can be achieved by mechanical coupling of the motors. For example, the regulation of dynein activity by the local curvature of the flagellum can produce helical bending waves.⁹⁹ Another possible mechanism proposes that force on the dynein motors transverse to the axoneme axis is the key regulator of dynein activity.⁸⁸ Recent theoretical analysis suggests that helical bending waves can spontaneously arise from the self-organization of dynein motors and microtubules.¹⁰⁰

The archetype of eukaryotic swimming is the sperm cell (shown in **Figure 5**). The sperm cell is divided into two major regions, a head and a tail. The head of the sperm is a few microns in size (in humans, the head is typically 5–6 μm in length and 2.5–3.5 μm in width). The head contains the DNA and an acrosomal vesicle, which contains hydrolytic enzymes that are thought to help the sperm penetrate the egg's outer

coat.⁸⁶ The tail includes a midpiece and the flagellum. The midpiece connects the flagellum to the head and also contains mitochondria. The flagellum is comprised of the axoneme surrounded by a plasma membrane. In mammals, the flagellum can be well over 100 μm in length.⁸⁸ In addition, some sperm, such as the sperm cell from mammals, also have nine outer dense fibers that surround the axoneme and are presumed to increase the stiffness of the flagellum.⁹⁵ As mentioned previously, the swimming of sperm is driven by traveling bending waves that move along the flagellum (**Figure 5(a)**). The waves are not sinusoidal, but rather appear to be composed of circular arcs connected by short straight regions.⁹⁷ Four parameters determine the shape of these traveling waves: the frequency, the radius of curvature in the bent regions, the wavelength, and the amplitude.⁹⁷ In sea urchin sperm, the frequency is observed to be around 30 s^{-1} , average radii of curvature are around 5 μm , the wavelength is around 20 μm , and the amplitude is around 4 μm .⁹⁷ Sea urchin sperm swim at 100–160 $\mu\text{m s}^{-1}$. In comparison, hamster sperm have a wavelength of around 160 μm , an amplitude of 23 μm , a frequency of 11 s^{-1} , and swim at velocities of 160 $\mu\text{m s}^{-1}$.¹⁰¹ The frequency measured in bull sperm is about 15 s^{-1} .⁹⁵ Some of the differences between the sea urchin parameters and the mammalian sperm parameters may be due to increases in the stiffness of the flagellum caused by the outer dense fibers.⁹⁵

As mentioned at the beginning of this chapter, the environment matters. Increasing the viscosity of the surrounding fluid increases the resistive force that the dynein motors have to work against to bend the flagellum. In hamster sperm, increases in the external fluid viscosity lead to decreases in the amplitude and wavelength of the flagellar waveform and also decrease the swimming speed.¹⁰¹ Mammalian sperm have to move through the female reproductive tract, some of which is filled with cervical mucus, a viscous polymer-filled fluid that responds to applied force with both solid-like and fluid-like behavior (i.e., it is a viscoelastic fluid).^{102,103} In addition, surrounding the egg are two zones known as the cumulus oophorus and the zona pellucida.¹⁰⁴ The cumulus oophorus is a layer of cumulus cells embedded in a viscoelastic extracellular matrix, which they have secreted.¹⁰⁴ The zona pellucida is a glycoprotein layer of extracellular matrix that lies just outside the plasma membrane of the egg. For fertilization to occur, a sperm cell must pass through both of these regions. A freshly ejaculated sperm cell typically swims with a fairly symmetric waveform and moves along a mostly straight path.^{105–107} In the female tract, though, the sperm undergo a change in their motility, producing larger-amplitude, more asymmetric beats¹⁰⁸ (**Figures 5(b)** and **(c)**). This transition is known as hyperactivation and is presumed to facilitate motility through mucus-like environments. Indeed, hamster and mice sperm that are hyperactivated are more effective at penetrating viscoelastic media.^{101,109}

There is a fascinating, though most likely coincidental, analogy between eukaryotes and prokaryotes: Both of these cell types swim using a filamentary object called a flagellum. In addition, we saw that a group of bacteria, the spirochetes, of which some members are highly pathogenic, causing diseases such as Lyme disease, syphilis, and leptospirosis, move via flagella that are enclosed within the periplasmic space in close contact with the cell body. Interestingly, a group of parasitic

eukaryotes, the trypanosomes, have a single flagellum attached to the cell body that drives motility. *Trypanosoma brucei* is the causative agent of African sleeping sickness.^{110,111} The cell body of *T. brucei* is approximately 20 μm long and has an extended tear drop shape, having a relatively large posterior section that tapers into a long, narrow anterior region.¹¹² A single flagellum is attached to the cell body along its length. Recent observations of the swimming of *T. brucei* using high-speed time-lapse imaging revealed that rather than propagating traveling-wave deformations like other eukaryotic flagella, the *T. brucei* flagellum propagates helical regions of opposite chirality separated by kinks,¹¹² which is surprisingly reminiscent of the swimming of *Spiroplasma* (as described in the previous section). These traveling kinks propagate from the posterior end of the cell to the anterior at speeds of 85–140 $\mu\text{m s}^{-1}$, and propel the cell at velocities of 5–10 $\mu\text{m s}^{-1}$.¹¹² It is intriguing that eukaryotic and prokaryotic invasive pathogens use such similar methods.

Where sperm cells and trypanosomes use a single flagellum to drive their motility, other cells, such as *Chlamydomonas reinhardtii*, use two flagella. *Chlamydomonas* is a single-celled alga that is commonly found in soil and water and is also one of the major model systems for studying flagellar dynamics.¹¹³ These cells are roughly spherical with a length of about 10 μm ^{114,115} (Figure 6(m)). Two flagella, each roughly 12 μm in length, extend from one end of the cell.¹¹⁴ During swimming, the flagella beat at ~ 50 Hz in a mostly synchronous manner that is reminiscent of a breaststroke, and with one flagellum slightly lagging the other¹¹⁴ (Figure 6). During swimming the cell body rotates about the body axis two times per second.¹¹⁶ The two flagella are labeled *cis* and *trans* according to their position relative to the eye spot, a light-sensing organelle used for phototaxis (cells swim towards the light in order to use photosynthesis for energy production, but can also move away from light),¹¹⁴ and there is some reason to believe that the flagella may be slightly different, as flagella that are removed from the cell body have different beat frequencies.¹¹⁷ In order to move toward light, *Chlamydomonas* cells increase the beat frequency of the *trans*-flagellum and decrease the frequency of the *cis*-flagellum,^{118–120} possibly by modulating cytoplasmic Ca^{2+} concentration.¹²¹ When *Chlamydomonas* cells are swimming in the dark, they are able to diffuse. This process was recently shown to be similar to the run-and-tumble behavior of bacteria, where *Chlamydomonas* cells tumble due to periods of asynchronous flagellar

beating¹²² (Figure 6(a)–(l)). Many algae, including *C. reinhardtii*, are also capable of swimming either up toward the surface of water or down away from the surface. This swimming is known as gravitaxis, as it uses the Earth's gravitational field to determine the correct direction to swim.^{115,123} Two separate mechanisms have been proposed for gravitaxis. The first suggests that if the center of mass is not at the center of the cell geometry, then gravity will torque the cell, which can act to align the cell in the proper direction.^{124–126} The other proposes that a sensor in the cell is responsible for determining the proper direction, possibly by a stress-activated channel in the cell membrane.¹²³

At the other end of the spectrum, some cells use a large number of short cilia to propel themselves. Possibly the best known representatives from this group are *Paramecium*. These cells can be as large as 500 μm long and 150 μm wide. The cell surface is completely covered in cilia. The cilia can be considered to lie in columns that run at a slight oblique to the long axis of the cell. During normal swimming, the cilia beat in a coordinate motion, with neighbors along the columns slightly out of phase with one another, while neighboring cilia in the rows perpendicular to these columns are in phase.¹²⁷ This coordination sets up traveling metachronal waves that drive the cell in the opposite direction at speeds ranging from 500 to 1000 $\mu\text{m s}^{-1}$.¹²⁸ The swimming path of the cell is a left-handed helix.¹²⁹ The beating of a single cilium is slightly different than that described for flagella. The ciliary motion can be broken down into a power stroke and a recovery stroke. During the power stroke, the cilium has a characteristic S shape and lashes forward in a direction roughly parallel to the metachronal wave direction.¹²⁷ During a recovery stroke, they curve and rotate counterclockwise close to the cell body surface.¹²⁷ Cells can modulate both the beat frequency and the direction of the ciliary power stroke in order to alter their speed and/or swimming direction.¹³⁰ The coordination of the cilia is mechanical, and changes in the viscosity of the environment can alter the coordination of the cilia as well as the swimming behavior of the cell.¹²⁹ Indeed, increasing the external fluid viscosity causes the swim path of the cell to change for a left-handed helix to a right-handed helix, and at high viscosities the cilia do not remain coordinated over the whole length of the cell.¹²⁹

Swimming requires a cell to exert force on the surrounding fluid. The reaction force then drives the cell through the fluid. It is therefore possible for the same apparatus that permits

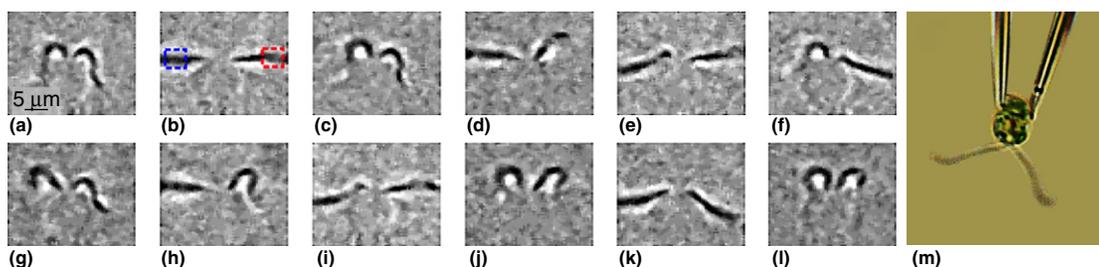


Figure 6 The swimming of *Chlamydomonas*. (a)–(l) The dynamics of the beating of *C. reinhardtii* flagella. The flagella beat in synchrony but occasionally slip out of phase with one another (as shown in panels (f)–(h)). (m) A single *C. reinhardtii* cell held by a micropipette. Images courtesy of R. E. Goldstein and originally published from Figure 1(a) and 2(a) in Goldstein, R. E.; Polin, M.; Tuval, I. Noise and synchronization in pairs of beating eukaryotic flagella. *Phys. Rev. Lett.* **2009**, *103*, 169103. Copyright by American Physical Society.

swimming to be used to pump fluid. Multicellular organisms often use cilia to do just that. For example, the clearing of mucus from the respiratory system is handled by cilia,¹³¹ and the left-right asymmetry in mammals is created by a counterclockwise flow driven by nodal cilia in the embryo.¹³² It is almost shocking how useful an axoneme can be.

Not all eukaryotic cells use axonemes to swim, though. Some cells change their entire cell shape in order to move. This process, known as metaboly, generates thrust force by shifting the internal components of the cell around, which deforms the contour of the cell in such a way that the cell is able to propel itself in a given direction.¹³³ The biophysical mechanisms underlying this unique motility are largely unexplored.

7.11.2.3 Modeling the Biomechanics of Swimming

In the preceding sections, the author was very cavalier with respect to the biomechanics of fluid-structure interactions. All too often statements were made that rotating helices, undulating filaments, and propagating kinks pushed on the surrounding fluid. Hopefully, you, the reader, were not overly doubtful and were willing to accept that these things could happen. In this section, these statements will be justified. Here, we will examine the fluid mechanics involved in cellular swimming and find that it is possible for a flapping or twirling appendage to propel a cell. But not only that, if the appendage is somewhat flexible, then the drag force from the fluid will also alter the shape of the appendage, which will affect the swimming speed, which changes the response force, thereby re-altering the shape, and so on. That is, fluid-structure interactions can be complicated. But the general principles are not, and that is what we will focus on. This discussion will be somewhat brief, but a very nice and much more complete review of the physics of microbial swimming is given in Ref. 134.

The behavior of fluids is size dependent. If you get into a swimming pool and push off against the sides of the pool with your legs, you will coast for a while and then eventually come to rest. The reaction force from pushing causes you to accelerate, due to your inertia you coast, and resistance from the fluid causes you to slow down and stop. If, instead, you were a cell, as soon as you were no longer in contact with the wall of the pool, you would stop. Effectively, cells have no inertia; the resistive force from the fluid is so large relative to the cell's inertia that the cell comes to rest instantaneously once the applied force is removed. Let's examine how this effect arises.

The movement of a fluid is governed by Newton's second law: Force is equal to the time rate of change of momentum. Since a fluid is a distributed object, we consider small-volume elements inside the fluid, that are large compared to the size of the fluid molecules, but small compared to the extent of the fluid. In each of these volumes we consider the density, ρ , and average velocity, \mathbf{v} , of the fluid molecules. The product of these two quantities is the local momentum density. From Newton's second law, the time rate of change of the momentum density is equal to the sum of the forces per unit volume that act on the volume element. These forces can be broken down into three components, two that are related to the interaction

between the fluid and itself, and the other that is due to external forces, such as gravity. The first of the internal forces is the viscous force. If neighboring fluid is moving at a different velocity, then it must slide past the nearby fluid. This sliding will produce a force that is proportional to the difference in velocity. The constant of proportionality, which we will denote by η , is known as the viscosity of the fluid. The second component is the pressure force. Fluids at a given temperature like to have a given density. If the motion of a fluid leads to compression or extension of the fluid away from this density, then there is a restoring pressure that tries to get the fluid back to its preferred density. A constitutive equation defines the relationship between the density and the pressure. However, for cellular swimming, most fluids are incompressible. In these cases, the pressure is determined to be whatever force is necessary to keep the fluid at fixed density. Putting all these effects together, we get a system of equations that defines the motion of an incompressible, Newtonian fluid (a Newtonian fluid is a fluid that has a viscosity that is independent of the velocity of the fluid):

$$\underbrace{\rho \left(\frac{d\mathbf{v}}{dt} + (\mathbf{v} \cdot \nabla) \mathbf{v} \right)}_{\text{inertia}} = \underbrace{\eta \nabla^2 \mathbf{v}}_{\text{viscosity}} - \underbrace{\nabla P}_{\text{pressure}} + \underbrace{\mathbf{f}}_{\text{external forces}} \quad [1]$$

$$\underbrace{\nabla \cdot \mathbf{v}}_{\text{incompressibility}} = 0$$

These equations are known as the Navier–Stokes equations. If we consider motions of the fluid on a characteristic length scale L with characteristic velocity U , then we can nondimensionalize the first of these equations using dimensionless length $\tilde{\mathbf{x}} = \mathbf{x}/L$, velocity $\tilde{\mathbf{v}} = \mathbf{v}/U$, time $\tilde{t} = Ut/L$, and pressure $\tilde{P} = PL/\eta U$:

$$Re \frac{d\tilde{\mathbf{v}}}{d\tilde{t}} = \tilde{\nabla}^2 \tilde{\mathbf{v}} - \tilde{\nabla} \tilde{P} + \frac{L^2 \mathbf{f}}{\eta U} \quad [2]$$

Here $Re = \rho UL/\eta$ is the Reynolds number, a dimensionless number that gives the magnitude of the inertial terms to the viscosity. For a fast swimming *Paramecium* of size $150 \mu\text{m}$ (it is appropriate to use the diameter not the length) swimming at $1000 \mu\text{m s}^{-1}$, the Reynolds number is approximately 0.15, and for most swimming cells, $Re < 0.01$. Therefore, as promised at the beginning of this section, even for the largest and fastest swimming cells, viscous forces dominate inertia, and the inertia of the fluid can typically be neglected. Ignoring the inertial terms in the Navier–Stokes equations leads to the Stokes equations:

$$\eta \nabla^2 \mathbf{v} - \nabla P + \mathbf{f} = 0$$

$$\nabla \cdot \mathbf{v} = 0 \quad [3]$$

This system of equations describes the motion of the fluid around the swimmer and also the force that the fluid exerts onto the swimmer. But how do we use it to determine the dynamics of a swimming organism?

Let us consider an idealized picture of a swimming bacterium, a spherical cell body connected to a single helical flagellum (Figure 7(a)). If the flagellum rotates at a fixed

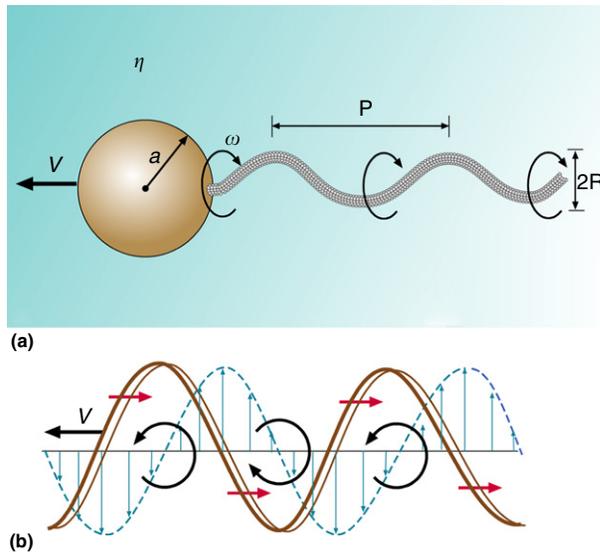


Figure 7 Simple models for swimming microorganisms. (a) An idealized bacterium swimming in a fluid with viscosity η . The cell body is considered to be a sphere with radius a . The fluid drag on the cell body is then $6\pi\eta a$. A single helical flagellum with helical radius R and pitch P rotates with velocity ω . The thrust force that is generated by this rotation must balance the drag force on the cell body, in order that the total force on the swimmer is zero. (b) The Taylor sheet. A sinusoidal wave of deformation travels to the right (as depicted by the brown solid lines and the red arrows). This traveling wave produces vertical fluid flow at the surface of the sheet (blue arrows and dashed line), and produces an alternating array of vortices in the fluid near the sheet (solid black lines). The vortical motion creates a net leftward fluid flow at the surface of the sheet that drives the sheet forward. This drawing is based on Figure 3 in Lauga, E.; Powers, T. R. The hydrodynamics of swimming microorganisms. *Rep. Prog. Phys.* **2009**, *72*, 096601. Copyright by IOP.

angular velocity ω , the cell swims at a constant velocity V in the direction parallel with the helix axis. (Here we are ignoring torques that can arise if the flagellum is not an integer number of turns or if the helix axis is not perpendicular to the cell body surface.) How do we use the Stokes equations to determine the magnitude of V ? The Stokes equations (eqn [3]) are a boundary value problem. If the velocity of the fluid is known at the boundaries of the fluid, then the fluid velocity can be calculated everywhere inside the boundary. An interesting property of fluids is that they effectively stick to surfaces. That is, the velocity of a fluid at a surface is equal to the velocity of the surface. This requirement is known as the no-slip boundary condition. Therefore, the velocity of the fluid at all points along the surface of the cell body must be V . The flagellum moves with the cell at velocity V , but also rotates, so there is an additional velocity that arises from this rotation. The fluid that is in contact with the flagellum moves with this same velocity. Therefore, we know the velocity of the fluid at all points on the surface of the bacterium up to a constant value. If we assume that outside the cell there are no external forces acting on the fluid, then the force density \mathbf{f} is zero at all points in the fluid. In addition, far away from the bacterium (at infinity), the fluid velocity should be zero. We can then solve the Stokes equations to find the fluid velocity everywhere.

Now how do we use that to find the magnitude of the swimming velocity? Here we take into account Newton's third law, twice. First, the force that the bacterium exerts on the fluid is equal and opposite to the force that the fluid exerts on the bacterium. Second, the bacterium cannot exert a net force on the fluid. For example, if the flagellum pushes against the fluid, the fluid will push back against the flagellum. The flagellum tries to move forward and pushes against the cell body. The cell body has to push back against the flagellum with an equal and opposite force, and the total force on both the flagellum and cell body therefore has to be zero. The force that the fluid exerts on the cell can be figured out by integrating the Stokes equations over the volume of the fluid:

$$\begin{aligned} \mathbf{F} &= \int (\eta \nabla^2 \mathbf{v} - \nabla P) dV \\ &= \int \left(\frac{\eta}{2} (\nabla \mathbf{v} + (\nabla \mathbf{v})^T) \cdot \mathbf{n} - P \mathbf{n} \right) dA \end{aligned} \quad [4]$$

The first integral is over the volume of the fluid and the second integral is over the surface of the bacterium. Here the superscript T stands for the transpose and \mathbf{n} is the normal direction to the bacterial surface, and Gauss's law has been used to convert the volume integral into a surface integral. The velocity that the bacterium swims at is the velocity for which \mathbf{F} is equal to zero.

What is described above is a sketch of how one might calculate the swimming velocity of a bacterium. Even for this highly simplified problem, to get the solution using this method requires discretizing the cell body and flagellar boundaries and solving the Stokes equations numerically everywhere outside those boundaries. Indeed, one of the major difficulties of modeling swimming at low Reynolds number is that the force that acts at a point on a swimmer is determined by the fluid velocity everywhere. So, it's not quite as simple as the above description makes it sound. There are other methods, though, that can also be used to solve these equations and determine the swimming behavior of a cell. In what follows, some of these will be briefly described.

The first question that one might be interested in asking is whether a given sequence of body movements will lead to net translation through a fluid. Purcell noted that because the Stokes equations do not depend explicitly on time that net movement is only possible if the cycle of shape changes that is used to swim is not symmetric in time.¹³⁵ Another way to say this is that if we were to film an organism trying to swim, the only swimming strategies that will work are those that look different when we watch the movie backwards. And, importantly, it also doesn't matter whether the motions are fast in one direction and slow in the reverse direction. Flapping your hand forward quickly and then returning it backwards slowly does not gain you anything because inertia does not matter. This restriction on swimming at low Reynolds number is known as Purcell's scallop theorem. A consequence of the scallop theorem is that a swimmer that can only deform with one degree of freedom (say a hinge, like on the shell of a scallop) cannot swim at low Reynolds number. Note that the bacterium mentioned above can swim. The flagellum is a helical object; it can be either a right-handed helix or a left-handed helix (as mentioned above, the normal form of the bacterial flagellum is a left-handed helix). If we film the

bacterium swimming, we see a left-handed helix rotating counterclockwise. Playing the movie backwards, we see a left-handed helix rotating clockwise. Since the movie looks different when we play it backwards, the bacterium is not precluded from swimming according to the scallop theorem.

Though the scallop theorem tells us whether an organism that deforms using a given sequence of shape changes can swim, it does not tell us how fast the organism will swim. The physicist and mathematician G. I. Taylor performed the first calculations to determine swimming speed at low Reynolds number.¹³⁶ As already discussed, mammalian sperm swim by propagating a traveling wave along their flagellum. Taylor sought to simplify this swimming strategy in order to figure out a rough estimate for how fast a sperm cell could swim. He considered a thin, flexible, infinite sheet that propagated a sinusoidal traveling wave (Figure 7(b)). That is, consider a sheet that lies predominantly parallel with the xy plane. How fast will the sheet move if it undulates its position in the z direction as $z(x,y) = A \sin(kx - \omega t)$? If the amplitude of this wave (A) is treated as being small, then the Stokes equations can be solved as an expansion in A (the details of this calculation can be found in Taylor's original paper¹³⁶ or another nice discussion is presented in Ref. 137). The oscillations of the sheet set up a periodic array of vortices in the fluid, which create a net fluid flow at the surface of the sheet (Figure 7(b) and Ref. 134). This fluid flow propels the sheet with a leading order translational speed of $V = \frac{1}{2}kA^2\omega$ (i.e., it scales as the square of the amplitude times the wave speed $k\omega$). Taylor also applied a similar analysis to figure out the swimming speed of a long, thin filament that propagates traveling waves,¹³⁸ and Stone and Samuel modeled a ciliated organism as a sphere with traveling surface waves.⁸² Both of these analyses lead to similar results; the swimming speed scales proportional to the square of the amplitude of the wave times the frequency and inversely with the wavelength.

Most of the swimming organisms that we have considered use long, thin appendages or a long, thin cell body to generate thrust. Therefore, it is worthwhile considering the behavior of a very thin filament moving in a fluid. There are three primary ways that the motion of a filament in a fluid can be modeled: resistive force theory (RFT), slender-body theory (SBT), and immersed boundary methods (IBMs). All three of these methods are based on the same principle: the flow of a fluid caused by a force applied at a point in the fluid. If a single force \mathbf{F} is applied to a fluid at point \mathbf{x}' , then the Stokes equations become

$$\begin{aligned} \eta \nabla^2 \mathbf{v} - \nabla P &= \mathbf{F} \delta^3(\mathbf{x}') \\ \nabla \cdot \mathbf{v} &= 0 \end{aligned} \quad [5]$$

where $\delta^3(\mathbf{x}')$ is the three-dimensional Dirac delta function. These equations can be solved for the pressure and velocity to yield:¹³⁹

$$\begin{aligned} P(\mathbf{x}) &= \frac{\mathbf{F} \cdot (\mathbf{x} - \mathbf{x}')}{8\pi r^3} \\ v_i(\mathbf{x}) &= \frac{F_j}{8\pi\eta} \left(\frac{\delta_{ij}}{r} + \frac{(x_i - x'_i)(x_j - x'_j)}{r^3} \right) = \frac{F_j}{8\pi\eta} \mathbf{S}_{ij} \end{aligned} \quad [6]$$

Here $r = |\mathbf{x} - \mathbf{x}'|$, and the subscripts label the components in the x , y , and z directions. The tensor \mathbf{S} is the fundamental

solution of the Stokes equations and is known as a stokeslet. Since the Stokes equations are linear, the velocity field from a distribution of point sources is just a linear superposition of these stokeslets. It is also possible (and useful) to calculate the flows that arise from higher order point force distributions (such as a point dipole) (see Ref. 139). Indeed, note that many swimming cells use a moving appendage to generate thrust. For these cells, the cell body is passive, but still experiences drag from the fluid. Therefore, the location of the center of thrust and the center of drag are not coincident. Therefore, many swimming cells can be approximated as point dipoles.^{134,140,141}

Using analysis similar to what leads to eqn [6], it is possible to calculate the velocity of a thin, cylindrical rod of length L and radius a aligned along the direction \mathbf{t} that is acted on by a force \mathbf{F} :¹³⁴

$$\mathbf{v} = \frac{\ln(L/a)}{4\pi\eta L} (\mathbf{I} + \mathbf{t}\mathbf{t}) \cdot \mathbf{F} \quad [7]$$

with \mathbf{I} the identity matrix. In principle, a long, thin appendage, such as a bacterial flagellum, can be considered to be a sequence of these connected rods, and eqn [7] can be used to relate the local velocity of a segment of the filament to the local force per length ($\mathbf{f} = \mathbf{F}/L$). This procedure is known as resistive force theory, and the force is usually written in terms of the velocity as

$$\zeta_{\perp} \mathbf{v} + (\zeta_{\parallel} - \zeta_{\perp})(\mathbf{v} \cdot \mathbf{t}) \mathbf{t} = \mathbf{f} \quad [8]$$

where $\zeta_{\perp} = 4\pi\eta/\ln(L/a)$ and $\zeta_{\parallel} = 2\pi\eta/\ln(L/a)$ are the slender-body drag coefficients for motion perpendicular and parallel to the long axis of the filament respectively.¹⁴²⁻¹⁴⁴ It is interesting, and maybe surprising, that these drag coefficients differ by a factor of 2. It does not matter how long a filament is, it is always twice as easy to push it along its axis as it is to push it perpendicular to that axis. In addition, that these drag coefficients are not equal is extremely important for cell motility. If $\zeta_{\perp} = \zeta_{\parallel}$, then the restriction that the integral of \mathbf{f} equals 0 would make it impossible for a cell to swim using an undulating or rotating filament.^{142,145}

It is important to note that resistive force theory ignores the hydrodynamic forces that arise due to the motions of distal parts of the filament (which is only reasonable when distal parts of the filament remain at least a few radii away from each other). Though this restricts the applicability of this method, the simplicity of having a local theory that does not require the fluid velocity to be calculated everywhere makes RFT a very useful approximation for modeling the fluid dynamics of moving filaments.

A more accurate method for calculating the dynamics of a filamentary object in a fluid is slender-body theory, which seeks to calculate the nonlocal hydrodynamic interactions of distal parts of the filament as an expansion in the ratio of the radius to the length of the filament. Though a number of different methods have been proposed for carrying out these calculations,¹⁴⁶⁻¹⁵¹ the most intuitive was presented by Lighthill.^{144,152} Lighthill reasoned that a line distribution of stokeslets and point force dipoles provides an appropriate representation of the flow produced by a moving filament.

Therefore, the velocity of the fluid (and the filament) can be calculated using a Green function approach.

An alternative computational method for handling the motion of filaments immersed in a fluid was developed by Peskin, and is known as the immersed boundary method (a great review of this method is presented in Ref. 153). As in SBT, this method treats a filament as a sequence of point forces that act on the fluid. However, rather than calculating Green's function, the immersed boundary method discretizes the delta functions that define the positions of the points that comprise the filament. The Stokes equations can then be solved numerically to find the fluid velocity.

In order to use these methods to solve for the swimming of a cell, one needs to prescribe either the dynamics of the shape of the cell during swimming or the internal forces that act on the filament. The first of these is a kinematic description of swimming. The Taylor sheet and the swimming bacterium are examples of this method. The time-dependent shape of the cell defines the surface velocity of the swimmer. From this known surface velocity and using that the total force on the swimmer must be zero, it is possible to compute the swimming speed. This kinematic description has been used extensively – for example, to model the swimming of flagellated bacteria,^{154–156} *Spiroplasma*,⁷⁸ spirochetes,¹⁵⁷ sperm,¹⁴⁴ and artificial swimmers.^{135,140,145,158–163} In the other method, we assume that the internal forces are known and use this to compute the shape and the speed of the swimmer. This method is more problem dependent, as it requires knowledge about the internal mechanisms that are driving motility; however, the shape of the swimmer is determined by the resistive force from the fluid as well as the internal driving force. Therefore, this method allows one to study the changes in the shape that arise from hydrodynamic interactions, such as how a swimmer will respond to changes in the fluid properties¹⁶⁴ or how interactions between cilia or swimming cells can lead to phase locking.^{165–168} In addition, prescribing the internal forces allows one to explore whether a given internal mechanism can explain the motion of the cell (e.g., swimming sperm,^{88,99} ciliary rotation,¹⁰⁰ and spirochete locomotion⁶³).

In everything described above, the fluid was treated as a typical Newtonian fluid, such as water. However, in many biological situations, such as sperm swimming through vaginal mucus or a pathogenic bacterium invading an organ, cells have to maneuver through fluids that are filled with polymer and/or cells (i.e., they are non-Newtonian). Though most modeling efforts have ignored this complexity, a few groups have started to examine the effects that non-Newtonian fluid dynamics have on swimming. For example, if the fluid is treated as a Maxwell fluid (a linear constitutive relation where the fluid acts like an elastic solid on short timescales and like a viscous fluid on long timescales) and the shape undulations are defined, then it was shown that the swimming velocity (to leading order) is not affected by the non-Newtonian nature of the fluid.¹⁶⁹ However, taking into account second order effects using the nonlinear Maxwell model (or an Oldroyd B fluid model (for a detailed description of these non-Newtonian fluid models, see Ref. 170)), it was shown that the velocity of an infinite undulating filament decreases due to non-Newtonian fluid behavior,¹⁷¹ which agrees with an analysis of the

Taylor sheet in nonlinear, non-Newtonian fluids.¹⁷² When the internal filament force, rather than the filament shape, is defined, non-Newtonian fluids can lead to drastic changes in the shape and speed of a swimming filament.¹⁶⁴

7.11.3 Gliding and Crawling on a Surface

Getting around on a surface is quite different than moving through a fluid. There is nothing surprising here; we all know this quite well. In a swimming pool, you kick your feet and pump your arms, and your whole body slips through the bulk fluid. On a surface, though, we walk (or crawl) by pushing backwards with a leg (or arm) that remains fixed in position with respect to the surface, while lifting up the other leg and swinging it forward. Therefore, the basic process for moving on a surface uses localized adhesion (our planted foot) in conjunction with a de-adhered, sliding motion (the swinging foot). Cells do this too, except that cells don't have legs and feet. Well, at least most cells don't. There is one interesting cell that has something very analogous to feet. Since this walking type of surface-associated motility is probably the most familiar to us, we will begin our discussion with the wall-less bacterium *Mycoplasma mobile*.

7.11.3.1 Gliding Motility of Bacteria

Mycoplasmas are a genus of wall-less bacteria, with some members that cause disease. For example, *Mycoplasma pneumoniae* is the causative agent of walking pneumonia. Mycoplasmas are Mollicutes, like the Spiroplasmas, with the cell shape defined by the cell membrane and an internal cytoskeleton.¹⁷³ The Mycoplasmas are tiny, pear-shaped cells that are 0.6–0.7 μm in length and have a rounded cell body with a characteristic membrane protrusion at one pole of the cell, known as the 'nose'.^{174–176} Unlike Spiroplasmas, Mycoplasmas move in the direction of the nose on surfaces, such as plastic, glass, or the surface of epithelial cells.^{177–179} Hydrolysis of ATP is known to provide the energy for mycoplasmal motility.^{180–182}

One of the fastest and more studied of the Mycoplasmas is *M. mobile*, which glides smoothly at speeds of 2.0–4.5 $\mu\text{m s}^{-1}$.¹⁸³ The speed is temperature dependent and increases almost linearly by a factor of roughly 10 between 10 and 40 °C.¹⁸⁴ The stall force, though, is independent of temperature and is ~ 25 pN.¹⁸⁴ Observations of crawling cells suggested that the thrust force for motility of *M. mobile* was generated in the nose structure,¹⁸⁵ and electron microscopy later revealed a complicated 'jellyfish' structure in the nose.¹⁸⁶ This structure is approximately 235 nm long and 155 nm in diameter and has dozens of filaments extending from it. The filaments are covered in ~ 400 single protein particles that are roughly 20 nm in size and spaced at intervals of 30 nm.^{186–190} These proteins extend through the cell membrane and can interact with the substrate, and have therefore been proposed to act as legs. Based on these observations, a model was proposed where ATP binding to the cytoplasmic region of the protein legs induces a conformational change that exerts force against the substrate.¹⁹¹ In this model, the extracellular region

of the protein acts like a foot that can bind to the substrate via electrostatic interactions. The motor portion of the protein is connected to the foot by a flexible linker. Prior to ATP binding, the leg is extended forward and the foot is bound to the substrate. The conformational change in the motor domain upon ATP binding is a power stroke that helps pull the cell forward. At the end of this power stroke, the cell continues moving forward, being driven by the other legs, which act to produce tension in the linker region that helps break the adhesion between the foot and the substrate. Once the foot releases from the substrate, the leg slides forward due to elastic recoil and can then rebind to the surface, completing the cycle. Mathematical analysis shows that this model agrees well with the experimental measurements of the stall force and temperature dependence of the velocity.¹⁹¹ Therefore, the gliding motion of *Mycoplasma* may be quite similar to the walking of a centipede and exhibits a coordinated cycle of adhesion, thrust, and release that is characteristic of surface-associated motility.

Many other bacteria also glide across surfaces, including cyanobacteria, myxobacteria, flexibacteria, Chloroflexaceae, and Begiatoaceae,¹⁹² but the mechanisms are quite different than that of *Mycoplasma*. In fact, there are multiple mechanisms that bacteria use to glide, which isn't surprising as the definition of gliding motility is quite generic: translocation in the direction of the long axis of the bacterium when in contact with a surface.¹⁹³ Indeed, gliding motility exhibits a number of different characteristics (for reviews see Refs 194 and 195). For example, some species rotate during translocation,¹⁹⁶ while others do not. The cyanobacteria are some of the fastest gliders, moving at up to $10 \mu\text{m s}^{-1}$,¹⁹³ whereas species such as *Myxococcus xanthus* are only capable of going a few microns per minute.^{197,198} *Myxococcus xanthus* cells also leave behind them a trail of 'slime', where *Mycoplasmas* do not.

Myxococcus xanthus is probably the most studied of the gliding bacteria, due to its unique multicellular interactions. It is a common, Gram-negative bacterium that inhabits the soil.^{199,200} The cells are rod shaped with a diameter of about $0.5 \mu\text{m}$ and length around $5\text{--}7 \mu\text{m}$.^{201,202} When well nourished, cells live as individuals or as a swarm that pools individual cell's extracellular enzymes in order to feed collectively²⁰³ (Figure 8). In addition, when starved, the

colony congregates to form fruiting bodies, where some members sacrifice themselves to form the structure of the fruiting body, while others become dormant spores. During fruiting body formation, collective gliding of the cells modulated by cell-cell signaling leads to 'ripple' waves and cell streams.²⁰⁴

An interesting aspect of the gliding of *M. xanthus* is that it utilizes two genetically distinct mechanisms to glide. The first of these mechanisms is known as social (or S) motility. S-motility is mechanistically equivalent to 'twitching' motility in other bacterial species, such as *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*. It is driven by the extension and retraction of long filaments known as type IV pili^{205,206} (Figure 8(b)). These filaments are a helical arrangement of a single protein, pilin or PilA.^{207,208} The filament is $\sim 6 \text{ nm}$ in diameter and can be several microns long.²⁰⁵ Extension of the pilus is carried out by a number of proteins that include a nucleotide binding protein (PilB), a polytopic inner membrane protein (PilC), a prepilin peptidase and methylase (PilD), and an outer membrane protein (PilQ). One model suggests that pilin monomers are initially embedded in the inner membrane.²⁰⁵ PilD cleaves the pilin sequence, and the pilus forms with the help of other proteins, such as PilB, and extends out of a pore in the outer membrane formed by PilQ. After being extended, the pilus can adhere to the substrate or to nearby cells. Retraction of the pilus produces the force that drives S-motility (i.e., reeling in a pilus that is adhered to the substrate or to another cell acts to pull the bacterium forward). Retraction is driven by the protein PilT, which has the sequence of an AAA motor protein and includes a potential ATP binding site.^{205,209} In *N. gonorrhoeae*, it was observed that an extended pilus retracts at roughly $1 \mu\text{m s}^{-1}$ and retraction events occur sporadically every $1\text{--}20 \text{ s}$.²¹⁰ The force of retraction was also measured to be as large as 80 pN ; however, this may represent the force from more than one pilus.²¹⁰

The second mechanism for gliding in *M. xanthus* is known as adventurous (or A) motility. How A-motility works remains elusive. One possibility was suggested by experiments on the gliding cyanobacteria, *Phormidium uncinatum* and *Anabena variabilis*. Like myxobacteria, these cells secrete mucilage, or slime, while gliding. Hoiczky and Baumeister discovered that diluted India ink particles stuck to the secreted mucus, which

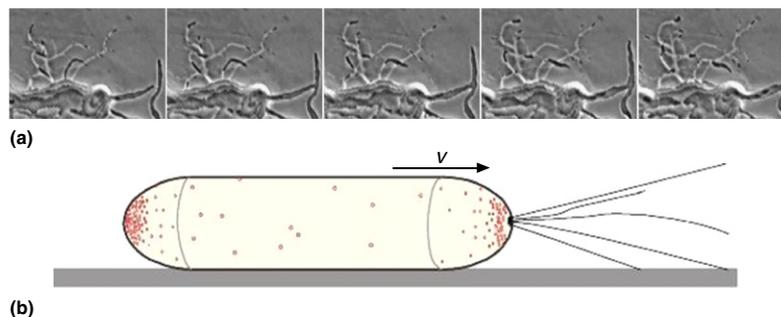


Figure 8 The gliding of myxobacteria. (a) Time series of the edge of a growing colony of *Myxococcus xanthus*. Some individual cells can be observed leaving the main swarm. Images courtesy of Roy D. Welch. (b) Schematic of an *M. xanthus* cell showing the type IV pili extending from the front of the cell and the nozzle-like structures that are distributed predominantly at the poles of the cell. These nozzles may extrude slime. Modified from Wolgemuth, C.; Hoiczky, E.; Kaiser, D.; Oster, G. How myxobacteria glide. *Curr. Biol.* **2002**, *12*, 369–377.

enabled them to visualize the secretion process using light microscopy.²¹¹ They found that the slime formed bands about the cell surface and, using fluid flow, were able to peel the bands away from the surface. The slime emanated from the cells at a rate of $3 \mu\text{m s}^{-1}$, which is comparable to the gliding speed. Electron microscopy had previously shown small pores, 14–16 nm in diameter, clustered around the septa in cyanobacteria.²¹² Hoiczky and Baumeister showed that these pores were part of a larger structure that was 70–80 nm in diameter and about 32 nm long,²¹¹ which is long enough to span the cell wall. The entire structure is called the junctional pore complex (JPC). It consists of an outer membrane pore complex attached to a channel ~ 13 nm in diameter that spans the peptidoglycan layer. The pore is both mirror and cylindrically symmetric with a bulge in the center. The ends are roughly 8 nm in diameter and the center bulge 14 nm in diameter. Ring-like structures encompass the pore near the central bulge. In both *P. uncinatum* and *A. variabilis*, the JPCs encircle the cell and are located near the septa. The channels formed by the JPCs are inclined at an angle of 30–40° relative to the cell axis, and are oppositely directed on either side of the septum. This angle provides directionality to the exuded slime that propels the cells forward. In *P. uncinatum*, the pores are aligned in a single row. In *A. variabilis*, several rows of pores line both sides of the septum. In some circumstances, prolonged cultivation leads to filaments that are nonmotile. These filaments do not secrete slime and the JPC organelles disappear, leaving behind only the trans-peptidoglycan channels.²¹³ Similar pores were later found in *M. xanthus*, and these pores were localized preferentially to the cell poles²¹⁴ (Figure 8(b)). In addition, slime was shown to be secreted from the ends of the cell in thin bands.²¹⁴ Therefore, slime secretion became a prime candidate for the mechanism underlying A-type motility.

But how can slime secretion push a cell? Slime is a polyelectrolyte gel – that is, a charged, crosslinked polymer mesh embedded in a fluid solvent. Such gels can swell to many hundreds of times their dry volume and can generate enormous swelling forces. Therefore, it is possible that slime is introduced into the pore in a deswelled state, perhaps deswelled by divalent cations (as in mucin granules²¹⁵). Fluid perfuses into the nozzle from outside of the cell and hydrates the slime, causing it to swell and extrude from the nozzle. As the gel stream leaves the nozzle it adheres to the substrate. This provides the swelling gel with a footing, allowing it to push the cell forward. Therefore, this surface-associated motility mechanism includes adhesion and sliding, but in a different way than walking. A mathematical model permitted calculation of the force produced within a single nozzle as the slime hydrates and expands. By multiplying by the number of observed nozzles at a cell pole, and comparing this force with an estimate of the drag on a cell, it was possible to show that slime extrusion could produce a propulsive force sufficient to account for the observed gliding speed of both myxobacteria and cyanobacteria, and to predict how the velocity of the bacterium depends on resisting forces.²¹⁴

Slime extrusion can explain a number of observed features of A-type motility,²¹⁶ such as the tendency for cells to follow one another,¹⁹² elasticotaxis,^{192,217} the velocity dependence on the volume fraction of the agar substrate,²¹⁸ and the etching of the agar by gliding cells.¹⁹² However, some more

recent experiments suggest that slime extrusion may not be the engine of A-motility. For example, *M. xanthus* cells that are treated with cephalixin become long filaments.²¹⁹ Filamentous mutants that lack S-motility (and therefore only possess A-motility) show that the speed of A-motility is independent of length,²¹⁹ and the leading pole of a curved filamentous cell moves forward without a commensurate motion of the rear pole.²²⁰ These two findings suggest that the motors that drive A-motility are distributed along the length of the cell. In addition, the A-motility protein AglZ is observed to form complexes that are stationary with respect to the substrate (i.e., they are presumed to be adhesion sites), and it has been proposed that an uncharacterized motor protein attaches to these adhesion complexes.²²¹ How a cytoplasmic motor might interact through the cell wall with a substrate-anchored extracellular adhesion, though, is not clear. A-motility remains mysterious.

So, there are up to four different ways that bacteria can glide: They can walk like Mycoplasma; they can extrude slime like cyanobacteria; they can pull themselves with pili; and they can use whatever mechanism drives A-motility. There is at least one more mechanism for gliding. The cytophagae are vigorous gliders that move at speeds of a few microns per second.¹⁹⁶ Like A-motility in *M. xanthus*, the speed of the cells does not depend on cell length.¹⁹⁶ However, cytophagae gliding shows a number of unique features that are not observed with cyanobacteria or myxobacteria. Small polystyrene latex spheres will move along the surface of the bacteria with speeds that are equivalent to the gliding speed. In addition, two spheres on the same cell often move in opposite directions. Furthermore, cells sometimes spin continuously about a pole at a frequency of ~ 2 Hz.¹⁹⁶ Gliding of *Flavobacterium johnsonae* (a member of the Cytophaga-Flavobacterium-Bacteriodes group) depends on the proton-motive force, and one of the proteins involved in gliding (GldA) exhibits a sequence similarity to ATP-binding-cassette transporters.²²² Two mechanisms have been proposed to explain the motility of *F. johnsonae*. First, because GldA resembles a transport protein, it has been suggested that a conveyor belt system could act like tiny tank treads, or coordinated lateral motion of outer membrane adhesion molecules has also been suggested.²²² Both of these mechanisms are highly speculative. It will require more experimental investigation, possibly coordinated with biophysical modeling, to determine what the actual mechanism for gliding is in these bacteria.

7.11.3.2 Eukaryotic Cell Crawling

Oddly enough, our discussion of the movement of eukaryotic cells on surfaces will begin with a bacterium, *Listeria monocytogenes*, the pathogen that causes listeriosis, a food-borne disease that causes gastroenteritis, encephalitis, meningitis, septicemia, and miscarriage.²²³ During infection, these bacteria invade nonphagocytotic cells. Once inside the cell, *L. monocytogenes* gets pushed around at speeds of around $10 \mu\text{m min}^{-1}$ by a comet-like structure that assembles behind the bacterium.²²⁴ These comets are formed from the actin protein inside the eukaryotic cell (i.e., *L. monocytogenes* hijacks the host cell's machinery in order to move). In fact, actin is one

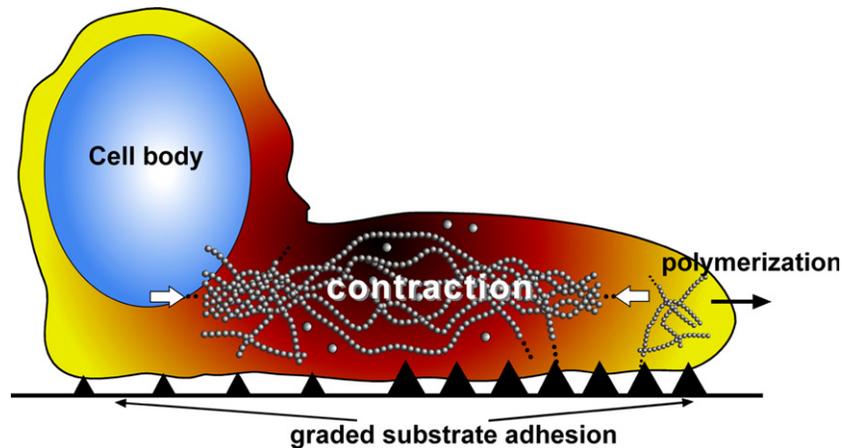


Figure 9 Schematic of a crawling eukaryotic cell. Polymerization at the leading edge pushes out the front of the cell. Adhesion to the substrate allows the cell to gain traction, which serves two purposes. First, it provides a firm anchor for the polymerization to push off, allowing the front of the cell to go forward. Second, it holds the rear front of the cell in position so that contraction of the cytoskeleton can pull the rear forward. The adhesion is graded, being stronger at the front of the cell and weaker at the rear, which facilitates the anchoring at the front of the cell and the sliding forward of the rear. Modified from Zajac, M.; Dacanay, B.; Mohler, W. A.; Wolgemuth, C. W. Depolymerization-driven flow in nematode spermatozoa relates crawling speed to size and shape. *Biophys. J.* **2008**, *94*, 3810–3823.

of the primary proteins responsible for moving eukaryotic cells that are in contact with substrates, and the process by which these comet tails form is directly related to the mechanism that crawling eukaryotes use to move across substrates.

Many eukaryotic cells can crawl when in contact with a solid or semi-rigid substrate. This type of motility plays a fundamental role during the development of an organism and during the progression of and immune response to disease. For example, nerve axons spread to make new connections in the developing brain, fibroblasts crawl during wound healing, metastatic cancer cells migrate to invade distant parts of the body, and neutrophils track down pathogens in our bodies. All of these cellular movements are driven by a complex network of proteins inside the cells that convert chemical cues into a biophysical response. Interestingly, unlike the gliding motility of the prokaryotes, the basic biomechanical process of crawling is very similar for all cells. Indeed, the standard model for crawling includes three or four basic physical processes: protrusion at the front of the cell; adhesion to the substrate; translocation of the bulk of the cell; and release of the rear^{225–227} (Figure 9). In some cells, these last two processes (translocation and release, which we will generically call retraction in the rest of this chapter) are coupled into a single motion.

Though the fundamental biomechanical processes are shared amongst crawling cells, the overall crawling behavior of cells can be markedly different between cell types. A crawling fish keratocyte has a characteristic half-moon shape (Figure 10(a)). The front of the cell is a wide, thin lamellipodium that is roughly 300 nm high, 40 μm wide, and 10–20 μm long.^{228–230} The cells crawl steadily at tens of microns per minute.^{228,229} Neutrophils crawl at similar speeds;^{231,232} however, they have a dynamic pseudopod that is longer than it is wide, being roughly 5 μm wide and 10 μm long.²³³ In addition, neutrophils *in vitro* only crawl along a given direction for about a minute before randomly reorienting.²³⁴ Fibroblasts, on the other hand,

are slow, moving at speeds of tens of microns per hour.²³⁵ The shape of a fibroblast is somewhat similar to a Y, with a somewhat triangular lamellipod connected to a thin tail.²³⁶

The primary force that drives the motility of these cells comes from the actin cytoskeleton, a network of crosslinked and entangled actin polymers. Monomeric actin (G-actin) is a globular protein that is approximately 5.4 nm in diameter. These monomers bind ATP and can polymerize and wrap into a linear, double-stranded, right-handed helix with a half-pitch of 36 nm.²³⁷ G-actin with ATP bound to it is more likely to polymerize than G-actin bound to ADP. The resulting actin polymer (F-actin) is polarized. Under physiological conditions, one end of the filament, known as the barbed end due to its appearance in electron microscope images, has a higher affinity for binding new monomers than the other end (pointed end). Upon binding, the ATP becomes hydrolyzed, and ADP-actin is more likely to dissociate from F-actin. Therefore, at the pointed end, disassembly dominates. F-actin can treadmill, with one end gaining monomers, the other end losing monomers, and the total filament length remaining constant. In addition, actin filaments can become capped, which stabilizes the filaments. F-actin can be severed and annealed. They can also be cross-linked. Though the biochemical interactions are complex (for a review, see Ref. 238 and references therein), the important features of the actin cytoskeleton are that it is capable of polymerizing, depolymerizing, and transmitting forces. These are the necessary qualities for achieving protrusion and retraction during crawling.

At the leading edge of crawling cells, membrane-bound proteins interact with cytosolic factors and lead to the nucleation and polymerization of F-actin. This polymerization is one mechanism for pushing the leading edge of the cell forward. But how does adding a monomer of G-actin to an F-actin filament push? The idea, originally proposed by Peskin et al. and known as a polymerization ratchet mechanism, is quite simple and elegant.²³⁹ Imagine a single actin filament

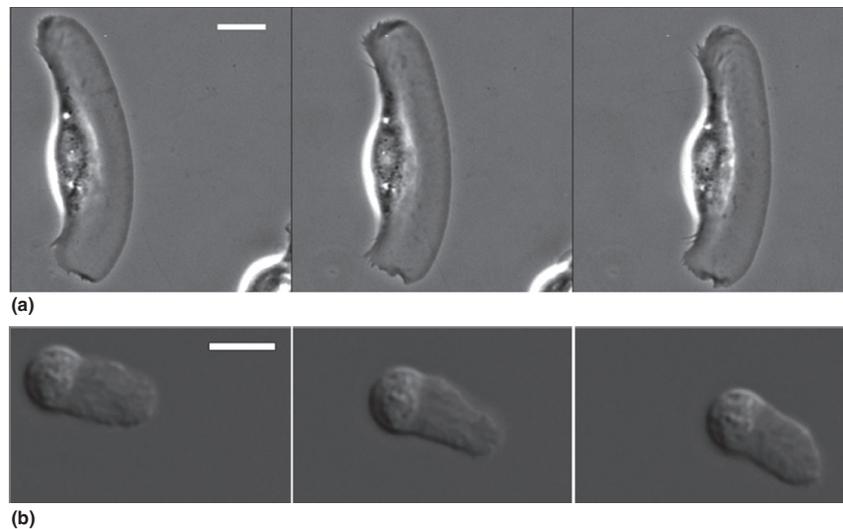


Figure 10 Two steady crawling eukaryotic cells. (a) A fish keratocyte moves steadily along a surface at speeds of around $10 \mu\text{m min}^{-1}$. The cell maintains a characteristic half-moon shape during crawling. Scale bar is $10 \mu\text{m}$ and there are 40 s between images. Images courtesy of K. Keren. (b) A crawling sperm cell from the nematode *Caenorhabditis elegans*. The cells are typically elongated in the direction of motion and move at speeds of up to $30 \mu\text{m min}^{-1}$. Scale bar is $5 \mu\text{m}$ and there are 30 seconds between images.

with its pointed end adhered to a wall and the barbed end in contact with a bead. Thermal fluctuations of the bead will move the bead away from and toward the actin filament. If a thermal fluctuation attempts to push the bead into the filament, then actin will prevent the bead from moving. In addition, when the bead is in contact with or very near the barbed end, polymerization will be sterically hindered. Therefore, polymerization will only occur when a fluctuation opens up a large enough gap between the bead and the filament. Once a monomer polymerizes, though, the bead will not be able to move back as far. Therefore, the position of the bead will be ratcheted by polymerization. Thermal fluctuations are smaller for larger beads, since the fluid drag force is larger. Actin polymerization is therefore slower the larger the bead is. Likewise, if a force is applied to the bead that opposes the bead's motion, the actin polymerization will be slowed down. This reasoning suggests that a single actin filament can withstand forces in the piconewton range.²³⁷ If we substitute an *L. monocytogenes* bacterium for the bead and anchor the single actin filament to the actin network in a cell, then we see that this thermal ratchet model could explain the motion of *Listeria*. However, there are some details that are missing. In reality, the actin filaments can fluctuate too. And they can also adhere to the surface of the bacterium. A modification of the polymerization ratchet mechanism that takes into account these details is known as the thermal elastic ratchet model, and it is able to explain the force–velocity relationship of actin comet tails.²⁴⁰ It may also be that polymerization of the actin about the bacterium produces stress in the comet tail that propels *Listeria* forward.²⁴¹ At the leading edge of a motile cell, thermal fluctuations of the membrane may also play a role in polymerization-driven protrusion.²⁴²

The leading edge of cells may also be pushed forward by blebbing. Cell blebbing is observed in many cell types during a wide range of processes, such as cell motility,²⁴³ apoptosis,²⁴⁴ mitosis,^{244–246} and development.^{247,248} Blebs are well

characterized, spherical protrusions that lack visible organelles. Blebs occur when a patch of cell membrane detaches from the actin cortex.²⁴³ Bleb formation has been shown to require both actin and myosin II.^{249,250} Recent work showed that blebs are caused by hydrostatic pressure gradients inside the cell. Experiments suggest that myosin II contraction in the actin cortex causes a localized increase in the cytosolic pressure, which then pushes the cell membrane away from the cortex.²⁵¹ These experiments showed that bleb expansion is opposed by osmotic pressure and membrane tension.²⁵¹ In *Dictyostelium discoideum*, it was observed that cell body retraction and overall cell displacement are reduced under conditions that prevent blebbing,²⁴³ which suggests that blebbing and polymerization contribute to leading edge protrusion in crawling amoebae.

For polymerization to push the leading edge forward, the actin network needs to be anchored to the substrate. Focal adhesions or adhesion complexes serve this function. These complexes are conglomerations of a large number of different proteins. Some of these proteins, such as integrin, are able to span through the cell membrane and bind to the substrate. On the cytosolic side of the adhesion complex, proteins link to the actin cytoskeleton, thereby allowing the actin cytoskeleton to gain traction with the substrate (a requisite for surface-associated motility). Recent work by Horwitz and co-workers has shown that substrate adhesions in migrating cells begin as small, dynamic adhesions at the leading edge of the cell.²⁵² These nascent adhesions colocalize with integrins and contain molecules commonly associated with adhesions. Many of these nascent adhesions undergo rapid assembly and disassembly, and the kinetics of these processes have been measured.²⁵² Some of the nascent adhesions, though, mature by growth and elongation at the lamellum–lamellipodium interface, recruiting new proteins, such as paxillin, talin, and vinculin, as they develop. Mature focal adhesions have grown under applied force.²⁵³ Therefore, it is likely that a factor in

determining whether an adhesion matures or disassembles is the force distribution in the actin cytoskeleton. At the rear of the cell, adhesions are weakened and are broken by forces generated in the cytoplasm. The dynamics of the adhesions lead to graded adhesion with the substrate. At the leading edge, nascent adhesions are weak, which allows actin polymerization to push forward. Mature focal adhesions are strong throughout most of the lamellipodium, which provides the anchoring required for motility. And adhesion is weak again at the back of the cell, allowing the rear to get pulled forward.

So how does the rear of the cell get pulled forward? The leading candidate for the force production that leads to hauling the cell forward is myosin, a family of molecular motors that can 'walk' along actin filaments. Each myosin molecule is capable of exerting a force of a few piconewtons against an actin filament.^{254,255} Indeed, myosin causes the actin filaments to slide with respect to one another and contracts our muscles. As myosin is also present in most motile cells, Huxley proposed that walking of myosin on bundled actin in a crawling cell produced the necessary force for movement.²⁵⁶ For this mechanism to work, the actin network needs to be able to convey force from myosin contraction in one region of a cell to distal points, and, indeed, the actin network of the cell is viscoelastic.^{257–261} On short timescales, the actin cytoskeleton behaves like an elastic solid with Young's modulus of around a few hundred pascals, which is what is expected for a network of semi-flexible filaments with a persistence length of a few microns.²⁶² On timescales of over a second, though, the actin network behaves more like a viscous fluid. Therefore, myosin activity in one region of the cell could be transmitted through the viscoelastic cytoskeleton. However, it is not clear whether myosin is the driving force for crawling. For example, myosin II-null *Dictyostelium discoideum* cells are still capable of translocating,^{263,264} and myosin IIA-deficient fibroblasts migrate faster than wild-type cells.²⁶⁵ In *D. discoideum*, it appears that the crosslinking of actin by myosin is more important than the motor activity.²⁶⁶

If myosin is not required for crawling, what other mechanisms could haul the cell forward? Two possibilities are membrane tension and depolymerization. Fish keratocytes are observed to maintain their area while crawling.²⁶⁷ The mechanism that maintains the area is unknown. Cell speed in keratocytes also correlates with cell shape in a way that can be described by actin polymerization at the leading edge.²⁶⁷ Taken together, these results suggest that membrane tension hauls the rear of a keratocyte forward. Another possibility is that the force is produced by the depolymerization (or disassembly) of the cytoskeleton. Indeed, depolymerization is necessary in order to provide a supply of monomer to the leading edge; that is, if the cytoskeleton kept polymerizing, but never fell apart, the cell would eventually use up all the available monomer for building new cytoskeleton at the leading edge. And, in one unique cell, depolymerization is the leading candidate for producing the force for crawling. The sperm cells from nematodes (which are also known as roundworms) do not swim; they crawl at speeds of tens of microns per minute (**Figure 10(b)**). At a qualitative level, the crawling is identical to that of other crawling eukaryotes: Cells protrude at the front, adhere to the substrate, and retract at the rear; however, nematode sperm utilize a cytoskeleton

composed of major sperm protein (MSP), instead of actin.²⁶⁸ MSP is quite different than actin. It forms apolar filaments and does not bind ATP.²⁶⁸ In addition, there are no molecular motors that have been identified that bind to MSP. Therefore, an acto-myosin contraction is not involved in the retraction of the cell rear. *In vitro* experiments using cell-free extracts from *Ascaris suum* (a species of nematode) sperm show that depolymerization of the MSP network induces contraction of the cytoskeleton.²⁶⁹ But how does depolymerization produce force? One possibility is that removal of polymer filaments from an existing network provides more space for the remaining filaments to fluctuate. The more a filament fluctuates, the closer its ends get to one another, and therefore a network of filaments can contract when some filaments are removed. A mathematical model based on this idea showed that this mechanism can produce sufficient force to explain the *in vitro* data,²⁷⁰ and later it was shown that depolymerization-driven contraction can also explain how crawling speed depends on the size and shape of nematode sperm cells.²⁷¹ These results strongly implicate depolymerization as the force-producing mechanism for retraction in nematode sperm motility.

An interesting feature of many crawling cells is that they can sense and respond to the stiffness of the surrounding environment, a capability known as mechanosensing. It was noted over 25 years ago that fibroblasts that were plated on glass were more spread and less elongated than fibroblasts grown in three-dimensional collagen matrices,²⁷² and cells that were grown on square adhesive islands showed stress fiber formations have actin filament bundles that lie along the diagonals of the square cell.²⁷³ More recently, it has been observed that cell proliferation can be affected by substrate stiffness.^{274–277} Cell motility is also affected by substrate stiffness. For example, fibroblasts migrate slower on stiff substrates than they do on soft ones;²⁷⁸ however, directed motility is more persistent on stiff substrates than on soft ones.²⁷⁹ Even more surprisingly, when fibroblasts encounter a boundary between a hard substrate and a soft one, they behave differently depending on which side of the boundary they started on.²⁷⁹ Cells on the soft side of the boundary will move into the hard region, whereas cells that are on the hard side of the boundary will either move along the boundary or crawl away from it. Furthermore, cells can actually adjust the stiffness of their cytoskeleton in order to try to match the surrounding environment.²⁸⁰ These abilities are presumed to play a role in how cells respond when they are in different parts of the body, as tissue stiffness varies in different parts of the body.²⁸¹ However, how cells achieve this feat is still largely unknown.²⁸²

The last cell that we will discuss here is a unique eukaryote. It is a cell that can glide across surfaces at surprising speeds of up to $30 \mu\text{m s}^{-1}$ and seems to use an axoneme to do it!²⁸³ *Peranema tricophorum* cells are roughly $40 \mu\text{m}$ long and $10 \mu\text{m}$ in diameter. One flagellum extends out from the front pole of the cell, and another is attached to the undersurface of the cell body. The proximal portion of the extended flagellum (roughly two-thirds of the total length) remains straight and is adhered to the substrate during gliding, while the distal portion beats actively. Removal of the distal portion of this flagellum does not affect the gliding motility of *Peranema*, but

gliding speed is strongly dependent on the length of the adhered proximal region. In addition, beads that are adhered to the anterior flagellum translocate. How this flagellum produces this directional force on the substrate and adhered beads is not understood. Furthermore, the second flagellum probably plays some role in motility, as cells with the anterior flagellum removed are still capable of gliding at speeds of a few microns per second.

7.11.3.3 Modeling Eukaryotic Cell Crawling

As we have seen, the crawling of cells requires the three processes of protrusion, adhesion, and retraction. A full model of the biomechanics of cell motility would therefore need to include the integrated action of all of these processes. To date, models typically focus on only one of these components at a time. Therefore, a model for protrusion may focus on the polymerization kinetics of actin, without describing the mechanical response of the actin network or handling the details of the adhesion to the substrate. Or a model might look at the behavior of focal adhesions under applied force in a stationary cell or consider the mechanics of retraction during crawling using crude descriptions of adhesion and protrusion. In this section, we will briefly lay out the biomechanics that describe each of these processes. A more detailed review of this topic is given in Ref. 237.

A polymerization ratchet mechanism is the leading candidate for how force is produced to push the front of a cell forward. At the front of a cell, the actin filaments are predominantly aligned such that their barbed ends are in contact with the membrane, with the actin filaments on average aligned at 35° to the membrane.²⁸⁴ Let us consider the kinetics of polymerization at the barbed end of the filament, when the concentration of G-actin is g . In the absence of applied force, actin monomers can be added to existing barbed ends at a rate $k_{\text{on}g}$, and actin filaments can lose monomers at a rate k_{off} . If the size of an actin monomer is δ , then the velocity of the end of the filament due to polymerization is

$$V = \delta(k_{\text{on}g} - k_{\text{off}}) \quad [9]$$

When the filament is in contact with the membrane, the membrane exerts a force f back on to the filament. This force acts to reduce the likelihood that the membrane or filament will fluctuate enough to allow a new monomer to polymerize onto an existing filament. The probability that a monomer can polymerize is reduced by a Boltzmann factor, where the work required to add a monomer is $f\delta$. To remove a monomer does not require any additional work to be done on the system. Therefore, the off rate is not affected by the force. These arguments suggest that the force-velocity relation for a polymerization ratchet is^{239,240}

$$V = \delta(k_{\text{on}g}e^{-(f\delta/k_B T)} - k_{\text{off}}) \quad [10]$$

The value of the force and whether it is a function of the velocity or not depends on what is assumed about the interaction between the filament and the membrane (i.e., it is model dependent). Evidence suggests that some of the actin filaments are attached to the membrane. Therefore, these

filaments can produce (or add to) the force that the membrane exerts on the actively polymerizing filaments. If it is further assumed that fluctuations of the filaments are the primary mechanism for inserting new monomer, then this leads to the tethered elastic ratchet model.²⁴⁰ It is reasonable to believe that this model may address the major conceptual issues involved in polymerization-driven protrusion, even if it does not encompass all aspects of the mechanism.

Of all the biomechanical aspects of cell crawling, substrate adhesion is the one where the least work has been done to develop a theoretical understanding. Indeed, many models for cell crawling treat adhesions as either a rigid connection to the substrate or as a source for drag between the substrate and the cytoskeleton. This latter viewpoint, that the gross effect of adhesions is a drag force that is proportional to the cytoskeletal velocity, seems arbitrary at face value. However, if we consider that adhesion to the substrate is mediated by proteins that can bind and unbind from the surface, then this approximation is reasonable. Leibler and Huse considered spring-like adhesions that probabilistically bind to the substrate and were able to reason that the effective resistance from the substrate is proportional to the velocity with an effective drag coefficient that scaled with the number of bound adhesion molecules, the strength of the spring, and inversely with the binding/unbinding rate.²⁸⁵ A more detailed analysis of this type of process has been done in regard to myosin binding in muscle,²⁸⁶ and it can be shown that these types of adhesions respond viscoelastically.²⁸⁷ A hybrid mechanism, where adhesions stick until a critical force is applied and then the cytoskeleton is able to slide with respect to the substrate (known as a stick-slip mechanism), is able to explain the experimental observations that the leading edge of crawling cells periodically retracts and extends in a substrate-dependent manner.^{288,289} Though not directly related to motility, Novak et al. were able to explain the dynamic localization of focal adhesions in cells plated on micropatterned surfaces using a model that considered the response of focal adhesions to applied stress from contracting bundles of actin.²⁹⁰

The mechanical properties of the cytoskeleton are responsible for conveying the forces from protrusion, focal adhesions, myosin activity, and membrane tension to the rest of the cell. It is also possible that the cytoskeleton itself, without these other effects, creates some of the force that helps haul the cell forward (such as is suggested by the depolymerization model for nematode sperm motility). Experiments show that the actin cytoskeleton acts like a viscoelastic continuum (see previous section). Models for cell motility and mechanics, though, have used models that treat the cytoskeleton as an elastic solid, a viscous fluid, a viscoelastic fluid, and a two-phase fluid.

A constitutive relation relates the stress in an object to the displacement of material points in the object (i.e., the strain). For example, in an elastic solid the stress and strain are typically assumed to be linearly related, and in a viscous fluid the stress is related to the time rate of change of the strain. Therefore, if the stress is known, then one also knows either the positions of the points relative to one another (solid) or the velocities of the points relative to each other (fluid). A viscoelastic medium responds with fluid-like and elastic-like responses. Typically, one considers a linearly elastic solid in

series with dashpot elements.^{291–294} One feature of these viscoelastic models is that they do not assume that the stress is instantaneously equilibrated. Therefore, the dynamics of the stress are treated on an equal footing with the chemical kinetics. In this framework, the relaxation of the stress occurs on a timescale τ . This timescale should be dependent on the viscous dissipation in the system, which is usually defined through a drag force that is proportional to the velocity. A general equation that encompasses all of these linear constitutive relations is the linear Maxwell model:

$$\tau \frac{\partial \sigma_{ij}}{\partial t} + \sigma_{ij} = \eta \left(\frac{\partial v_i}{\partial x_j} + \frac{\partial v_j}{\partial x_i} - \frac{2}{3} \left(\frac{\partial v_k}{\partial x_k} \right) \delta_{ij} \right) \quad [11]$$

where the stress in this equation is the deviatoric stress (the traceless component of the total stress) and η is the viscosity. The medium is usually considered to be incompressible, so that the divergence of the velocity is zero. Force balance using the deviatoric stress gives

$$\nabla \cdot \sigma - \nabla p = 0 \quad [12]$$

where p is the pressure. Given an initial condition on the stress and boundary conditions, these equations (deviatoric stress, incompressibility, and force balance) form a linear, time-dependent system of equations. A standard boundary condition defines a prescribed force that acts on the boundary, $\sigma \cdot \mathbf{n} = \mathbf{F}$, where \mathbf{n} is the normal vector to the boundary and \mathbf{F} is the force. Other possible boundary conditions define both components of the velocity or the pressure and the tangential velocity.

Many models have treated the mechanical properties of the cell as an incompressible, viscous fluid.^{295–297} This corresponds to setting τ equal to zero in eqn [2]. In this limit, the equation is not time dependent; therefore, the dynamics are governed by the temporal changes in the boundary conditions and the shape of the cell.

When the constitutive relation is that of a linearly elastic solid, as has been used in a number of models,^{298–300} stress is proportional to the displacement vector, \mathbf{u} , which is the limit of eqn [2] where τ goes to infinity and the ratio η/τ goes to the shear modulus of the elastic solid. In this limit, eqn [2] can be integrated with respect to time. The pressure is usually chosen to be proportional to the divergence of the displacement, and the force balance equation for the stress is then the same as before. The boundary condition in the presence of applied force is also identical. An alternative boundary condition is to define the displacement vector on the boundary. Therefore, similar methods can be used to solve this system of equations as were used for the viscous fluid equation. As in the fluid model, we assume that the elastic constants depend on the chemical concentration and are therefore spatially dependent. One assumption that is being made in both of these cases is that the stress equilibrates on a timescale that is much quicker than any other timescales present in the chemical kinetics.

A number of groups also suggest a two-phase description of the mechanical properties of the cell that treats the cytoskeleton as an elastic solid and the cytosol as a viscous fluid.^{251,271,301,302} Whereas the previously mentioned models treat the cytoskeleton and cytosol as a single unit, two-phase

descriptions allow the polymer and fluid components of the cell to move with different velocities. This method typically uses the polymer volume fraction, ϕ , to describe the local density of polymer in the cell. A continuity equation then defines the dynamics of the polymer:

$$\frac{\partial \phi}{\partial t} = -\nabla \cdot (\phi \mathbf{v}_p) + J(\mathbf{x}) \quad [13]$$

where \mathbf{v}_p is the velocity of the polymer and $J(\mathbf{x})$ is a source or sink term that describes polymerization or depolymerization. The polymer component of the cell can be treated as an elastic solid or a viscous fluid, and the cytosol is typically treated as a viscous fluid that interpenetrates the polymer. These constitutive relations define the force balance for each phase, and viscous drag between the two phases (polymer and fluid) couples the dynamics.

These four constitutive laws are quite different, and it would seem that making the correct choice when modeling cell motility would be crucial. However, no overall consensus has been reached. One major issue is that models for cell motility are still in their infancy. The reality of what is going on in a crawling cell is quite complex, even if we only consider the biomechanics, without worrying about the biochemistry. Indeed, most experiments have focused on cell migration on a two-dimensional substrate. This scenario is probably much less complex than what happens in many physiological conditions, such as migration through the three-dimensional extracellular matrix, where a cell must adhere to and degrade a polymer network as it moves. However, consensus on what is the correct biomechanical description of 2-D crawling is still lacking, and most modeling efforts focus on making fairly crude comparisons back to experimental data. To determine the proper description of a cell will require creating testable predictions for experiments that can delineate between these different descriptions.

7.11.4 Conclusions

Here ends our journey through the bewildering diversity of cellular motility. We have seen that environment is key in determining what mechanism a cell can use to move. In a fluid, cyclic undulations are the primary way that cells exert force against their surroundings. Typically, long, thin filaments are used to create these periodic movements. The drag anisotropy of a filament allows these motions to produce thrust, but surface undulations will also work. Why cells almost ubiquitously use filaments is not entirely clear. When cells are in contact with a solid substrate or a 3-D matrix of polymer, then it is necessary to coordinate adhesion to the environment with release in order to gain traction, yet still slip through the environment. On a substrate, the mechanisms that cells use to move are somewhat analogous to what macroscopic organisms use: Some cells walk like mammals, others crawl like snails, and some even pull themselves along like a jeep winching itself out of the mud.

The second consideration that a cell has to make is how it is going to generate the deformations that drive motility. For a small bacterium, the cell wall may be too stiff for the

bacterium to deform. Therefore, using a rigid rotating helix or extruding pili may be the easiest way to create force. On the other hand, larger eukaryotic cells can actively flap flagella or constrict actin cytoskeletons.

Though we have touched on many biomechanical mechanisms that cells use to move, the author is certain that there are some, if not many, mechanisms that were left off. If you find your favorite motile cell missing from this discussion, I am sorry. And if the mechanism is vastly different from any of the cells that were mentioned, I would love to hear about it.

This chapter has avoided discussion of the workings of some of the molecular motors, such as dynein and myosin, which are key players in eukaryotic cell motility. Molecular motors form a field of study unto themselves and were too much to cover within the scope of this chapter. In addition, the focus was on biomechanics and completely ignored the biochemistry that is intricately involved. A complete understanding of cell motility is not possible without consideration of these factors.

So if you found yourself stuck inside a capsule with only shutters to look out at the world, what would you do to move?

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