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TUTORIAL

An introduction to cell motility for the physical scientist

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Abstract
Directed, purposeful movement is one of the qualities that we most closely associate with living organisms, and essentially all known forms of life on this planet exhibit some type of self-generated movement or motility. Even organisms that remain sessile most of the time, like flowering plants and trees, are quite busy at the cellular level, with large organelles, including chloroplasts, constantly racing around within cellular boundaries. Directed biological movement requires that the cell be able to convert its abundant stores of chemical energy into mechanical energy. Understanding how this mechanochemical energy transduction takes place and understanding how small biological forces generated at the molecular level are marshaled and organized for large-scale cellular or organismal movements are the focus of the field of cell motility. This tutorial, aimed at readers with a background in physical sciences, surveys the state of current knowledge and recent advances in modeling cell motility.

Preface

'I can remember the scene vividly—a fish epithelial cell known as a *keratocyte* moving in slow motion across the microscope's field of view. After plucking a scale from a goldfish and letting it incubate in media overnight, I was watching a doggedly determined, self-contained protein machine crawl across the slide. Amazing. I'd had years of training in engineering and physical science, and nothing we know how to build has that much functionality in such a small package. How does this cell move? How is it controlled? What is its role in preserving health? Most students of cell biology arrive at these questions as undergraduates or earlier, but it took me a little longer. After several degrees investigating and manipulating inanimate materials, I was attempting to learn basic biology as a postdoc. The process had its share of frustrations—experimental uncertainties, cell-to-cell variability, different sets of assumptions—but the elegance and robustness of biological systems never cease to amaze me. Exciting opportunities await those willing to embrace the challenge of learning a new field and applying the experimental and analytical tools of physical science to biological questions. Quantitative biology is alive and growing, and the potential impacts on health, disease, and technology are enormous. There is no time like the present to peer into the microscope and see what's crawling by.'—DAF

1. Biological and physical importance of cell movements

Problems in the movement of cells and their internal components are the topic of a great deal of investigation, because of the intrinsic interest of the processes and because of their medical importance [1]. Most cancers are not life-threatening until they metastasize and spread throughout the body. Metastasis occurs when previously sessile cells in a tumor acquire the ability to move and invade nearby tissues and circulate in the bloodstream or lymphatic system. A treatment that blocked the ability of tumor cells to acquire motility would largely prevent metastasis. The elaborate wiring of the human nervous system is generated during fetal

Table 1. Cell movements and their molecular mechanisms.

Cell movement	Cell structure needed	Molecular motor	Motor category
Movements through liquid			
Bacterial swimming	Flagella (bacterial)	Flagellar rotor (MotA/MotB)	Rotary
Eukaryotic swimming	Cilia, flagella (eukaryotic)	Dynein	Linear stepper
Metaboly	Unknown	Unknown	Unknown
Movements on solid surfaces			
Amoeboid motility (crawling)	Lamellipodia, filopodia, pseudopodia	Actin	Assembly/disassembly
		Myosin (several)	Linear stepper
Bacterial gliding	Junctional pore complex	Slime extrusion nozzle	Extrusion
Parasite gliding	Pellicle	Myosin (class XIV)	Linear stepper (probably)
Bacterial twitching	Type IV pili	Pilus base motor (PilT)	Assembly/disassembly? Linear Stepper?
Intracellular movements			
Chromosome segregation	Mitotic spindle	Kinesin (several), dynein	Linear stepper
		Tubulin	Assembly/disassembly
Organelle transport	Microtubule arrays	Kinesin (several), dynein	Linear stepper
	Actin gels	Myosin (class V, class VI, others?)	Linear stepper
	Actin comets	Actin	Assembly/disassembly
Rapid cell shape changes			
Muscle contraction	Sarcomere	Myosin (class II)	Linear stepper
Cytokinesis	Division furrow	Myosin (class II)	Linear stepper
Stalked ciliate recoil	Spasmoneme	Spasmin	Prestressed spring
Acrosome extension (*Thyone*)	Acrosomal vesicle	Actin	Assembly
Acrosome extension (*Limulus*)	Acrosomal bundle	Actin	Prestressed spring

development by the motile behavior of nerve cells, which send projections crawling along molecularly defined paths to connect peripheral body parts to the central nervous system. After spinal cord injuries, these connections are broken, but a medical treatment that encouraged nerve cells to reacquire the ability to produce motile projections might speed recovery and reverse paralysis. Defects in cell motility during fetal development are responsible for many common birth defects, including cleft palate and spina bifida. Other kinds of cell motility defects are responsible for a variety of conditions, ranging from male infertility to hereditary deafness to the susceptibility to lung infections seen in people with cystic fibrosis. Cell motility also underlies wound healing and the immune response.

The burgeoning field of nanotechnology offers another area in which detailed knowledge of the mechanisms of cell motility might prove useful. Cells have spent several billion years developing highly efficient machinery to generate forces in the piconewton-to-nanonewton range that operate over distances of nanometers to micrometers and function well in an aqueous environment. As we grow to better understand the mechanics of cell motility, we may be able to adapt the cell movement machinery for design and engineering purposes. Nanoscale sensors and actuators harnessing molecular motors could one day alert us to the presence of pathogens or guide delivery of drugs.

2. Types of cell movements

A wide variety of cell movements has been characterized by biologists and biophysicists to varying degrees of molecular and mechanistic precision (see table 1). Movements of whole cells can be roughly divided into two functional categories: swimming, or movement through liquid water; and crawling, or movement across a rigid surface. The physical problems faced by cells attempting to swim have been eloquently summarized by Purcell as 'life at low Reynolds number' [2]. Because viscous forces are many orders of magnitude greater than inertial forces at the speeds, viscosities, and length scales experienced by swimming cells, simple reciprocal motions cannot produce forward propulsion. Instead, most swimming cells use nonreciprocal motions of cell surface projections to swim. For bacterial cells, the rotation of a helical or corkscrew-shaped flagellum has been particularly well-characterized from biological and physical perspectives [3]. The flagellum is a long filament constructed by the noncovalent polymerization of hundreds of identical protein subunits, called *flagellin*. The speeds of flagellar swimming range from about 10 to 100 micrometers per second. Some bacteria such as *Vibrio cholerae*, the causative agent of cholera, have a single flagellum at one pole and swim rapidly. Others, including the common laboratory organism *Escherichia coli*, have multiple flagella distributed around their surfaces that gather together in a bundle during swimming and can fly apart in a regulated way when the bacterium chooses to change direction [4].

Rotary flagella have never been found in eukaryotes. Most swimming eukaryotic cells, ranging from human sperm to paramecia, use a second strategy, the propagation of a waveform down a flexible oar. When the cell surface projections used as oars are short and numerous, as on a paramecium, they are called *cilia*, and when they are long and few, as on sperm, they are called *flagella*. It is an unfortunate historical accident of terminology that bacterial flagella and eukaryotic flagella share the same name, as they

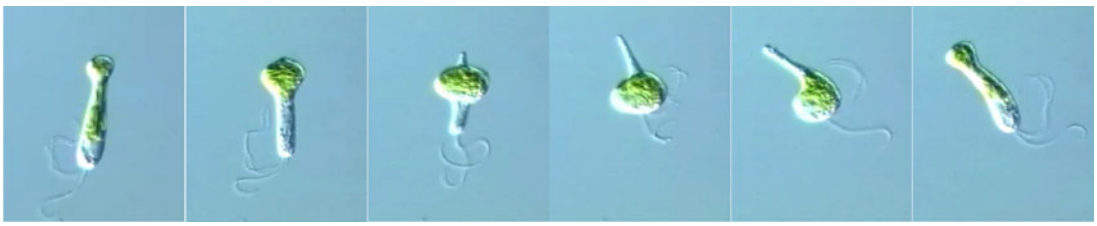

Figure 1. *Eutreptiella* movement through water. This single-celled organism shifts cellular contents to generate a local increase in drag that propagates from the front of the cell to the back, a process called *metaboly*. Still images from a video courtesy of Richard Triemer, Michigan State University.

are distinct structures. While bacterial flagella are filaments made of a single protein polymerized in a simple repeating unit, eukaryotic flagella and cilia have a complex structure involving a group of about 10 long parallel filaments that slide relative to one another and bend in a coordinated fashion, all surrounded by an extension of the cell's plasma membrane [5, 6].

The rotary corkscrews and flexible oars described by Purcell are not the only cellular strategies for swimming. Some species of *Euglena* and related protozoa (unicellular eukaryotes) swim by gradually changing the contour of their surface. This elegant movement, called *metaboly*, is the low Reynolds number equivalent of the high Reynolds number ice-skater increasing her rotational speed by pulling arm mass inward. In metaboly, the reshifting of cellular contents to generate a local increase in drag that propagates from the front of the cell to the back is sufficient to pull the cell's center of mass forward through the viscous aqueous environment (see figure 1). The *Eutreptiella* cell shown herein uses flagella and metaboly for movement, and some related species exhibit phototaxis (movement toward a source of light). Other protozoa offer similarly fascinating motility phenomena that await biophysical investigation.

Movement of cells across rigid surfaces can be achieved by an even greater variety of mechanisms than can swimming. The best characterized is amoeboid motility, or crawling motility, a general process shared by eukaryotic cells ranging from soil amoebae to human white blood cells. In amoeboid motility, a cell attached to a rigid substrate extends forward a projection at its leading edge that then attaches to the substrate. Long thin projections are called *filopodia*; flat veil-shaped projections, *lamellipodia*; and thick knobby projections, *pseudopodia*. All three types of projections are filled with assemblies of cytoskeletal actin filaments. After protrusion and attachment, the crawling cell then contracts to move the cell body forward, and movement continues as a treadmilling cycle of front protrusion and rear retraction [7]. The speed of amoeboid movement can range from less than one micrometer per hour to more than one micrometer per second, depending on the cell type and its degree of stimulation.

Although amoeboid motility is usually accompanied by significant cell shape changes, other forms of surface-based movement are not. The term *gliding* is used to describe several kinds of movement in which cells slide across a rigid substrate. For some bacteria, gliding appears to be driven by a low Reynolds number analogue of jet propulsion, in which a sticky and cohesive slime is extruded backwards to push the cell forward [8, 9]. Many eukaryotic parasites also perform a kind of gliding, but the mechanism is entirely different, relying on a complex dynamic organization of the pellicle, or skin of the cell [10]. Twitching motility is a special form of gliding seen only in groups of bacteria. Twitching bacteria throw out long pili, cell surface projections that resemble bacterial flagella except that they are straight instead of helical, that adhere to other nearby bacteria. The bacteria then retract the pili, pulling themselves toward their neighbors [11, 12]. In this manner, colonies of related bacteria growing on surfaces can organize themselves into a multicellular structure. The predatory bacterium *Myxococcus xanthus* prefers to hunt in packs and uses twitching motility to organize its hunting parties. Lonely *Myxococcus* switch to a slime-extruding form of gliding [13].

In addition to movements of the whole cell, as when a paramecium swims after its prey in a drop of pond water or a white blood cell in the human immune system tracks down an invading bacterium, important movements take place within the cell boundaries. One vivid example of this is the process of mitosis, in which the duplicated chromosomes in a dividing eukaryotic cell line up and are simultaneously segregated so that exactly one copy of each chromosome is delivered to each of the two daughter cells (see figure 2). A large, complex cellular structure called the *mitotic spindle* assembles and then segregates all of the chromosomes simultaneously [14]. Because of the necessity of accurate chromosome segregation to the life of all cells and the many ways in which this process can go wrong in the development of cancer, chromosome segregation has been a particularly well studied instance of intracellular biological motility [15].

Besides the chromosomes, many other large objects within the cell must be moved quickly to particular locations by directed intracellular transport. The need for directed transport rather than reliance on simple diffusion and trapping to localize cellular components is best imagined in large cells, such as neurons. The longest neuron in the human body has a single threadlike projection (the axon), a few micrometers in diameter, that reaches from the base of the spine to the foot, a distance of up to one meter. Most cellular components, including large organelles such as mitochondria, are synthesized within the cell body that lies in the spinal cord and must be delivered down the axon to the synapse, where the neuron forms an electrically active connection with the muscles that flex the toe. Generous estimates of the time it

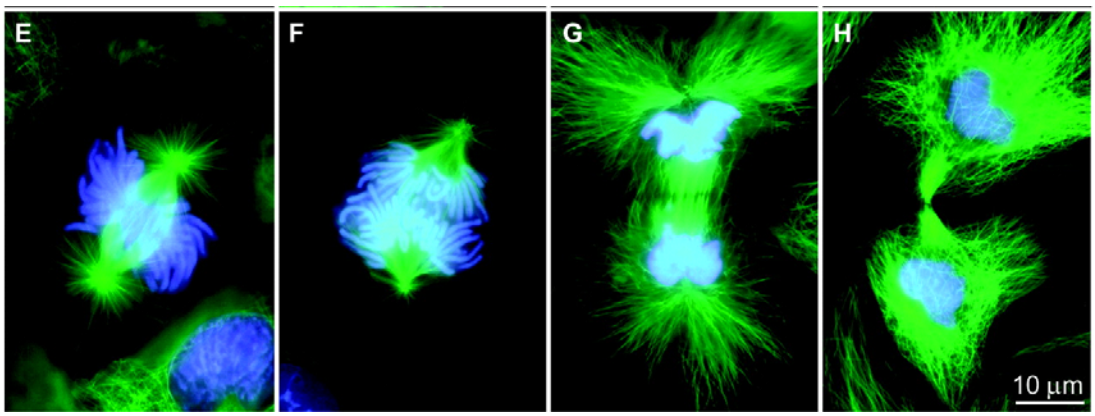

Figure 2. Fluorescence images of mitosis in fixed newt lung cells stained for microtubules (green) and chromosomes (blue). Reprinted with permission from C L Rieder and A Khodjakov, 'Mitosis through the microscope: advances in seeing inside live dividing cells', figure 3 E, F, G, H. 2003 *Science* **300** 91–6 (American Association for the Advancement of Science).

would take for a mitochondrion to diffuse that distance given the cell geometry range from 10 to 100 years. In fact, the mitochondrion can make the journey in just a few days, thanks to intracellular directed transport. Organelle transport is accomplished by a wide variety of mechanisms depending on the specific organelle involved [16]. The fastest known type of organelle transport is the racetrack-like circulation of chloroplasts in green algae such as *Chara*, which can achieve speeds greater than 60 micrometers per second [17].

A final important class of cell motility events comprises rapid and extensive cell shape changes. The best studied of these is skeletal muscle contraction, which has fascinated biologists and physicists for more than 200 years, since Galvani showed that electrical stimulation could cause the muscles of a frog's severed leg to contract [18]. The proposal in 1953 by Huxley that contraction may be due to the sliding of paired filament arrays relative to each other has proved to be largely correct, and most aspects of muscle stimulation and contraction are now understood in molecular detail [19]. This same type of filament sliding, using closely related molecular components, is responsible for the process of cytokinesis, when two daughter cells are pinched apart after chromosomes are segregated during cell division [20].

Organisms that lack skeletons and muscles may still exhibit rapid contraction, albeit by a different mechanism. A particularly striking case is the avoidance response of the stalked ciliate *Vorticella*, as originally described by van Leewenhoek [21]. When this timid tulip-shaped protozoan is touched on its belly, the stalk rapidly coils into a tight spring. With speeds approaching eight centimeters per second and force generation in the range of tens of nanonewtons, the contractile spasmoneme structure within the stalk is among the fastest and most powerful engines known in cell motility [22]. An opposite type of cellular shape change, rapid extension of a long stiff protrusion, is a common behavior of sperm cells that have contacted the jelly coat of a compatible egg. The rapid extension of this cell surface protrusion through the jelly coat is necessary to allow sperm and egg membrane fusion and proceeds by strikingly different mechanisms in different animal species [23, 24]. A more modest form of the same phenomenon is seen in the mating of the unicellular alga *Chlamydomonas* [25].

3. Types of molecular motors

Cells generally store chemical energy in two forms: high-energy chemical bonds, such as the phosphoanhydride bonds in ATP (adenosine triphosphate); and asymmetric ion gradients across membranes, such as the electrical potential seen in nerve cells. These sources of chemical energy drive all cell processes, from metabolism through DNA replication. The subset of cell proteins and macromolecular complexes that convert chemical energy into mechanical force are generally called *molecular motors*. Their astonishing variety reflects the diversity of cell movements necessary to life.

Known biological molecular motors may be divided into five general groups: (1) rotary motors, (2) linear stepper motors, (3) assembly and disassembly motors, (4) extrusion nozzles, and (5) prestressed springs. All of the various cell movements already described are performed by ensembles of molecular motors that fall into these categories (see table 1). Coordinated actions of many small individual components can give rise to large-scale (cellular or organismal) movements. Because the molecular motor appears to be the fundamental unit of biological motility, much experimental and theoretical effort has focused on understanding these motor elements.

The best characterized motor in the bacterial kingdom is the tiny rotary motor that enables bacteria to swim [3]. This motor uses ion flux down an electrochemical gradient to drive the rotation of the long, thin helical flagellum at a frequency of about 100 hertz. In *Escherichia coli* and its close relatives such as *Salmonella typhimurium* (a common cause of food poisoning), the motor can rotate in both directions, and switching from counterclockwise to clockwise rotation is under the control of an elaborate regulatory apparatus in the bacterium that detects whether it is moving toward or away from a desirable nutrient source [26]. Some other bacteria spin their flagellar motors in only one direction, but modulate the speed or the frequency of stops. All known biological rotary motors use energy stored in an ion gradient to produce torque.

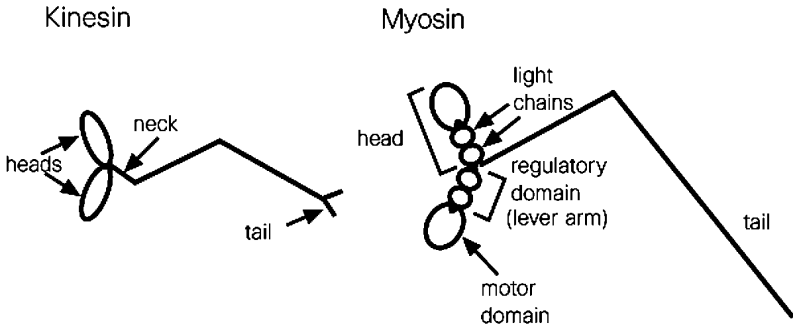

Figure 3. Schematic of conventional kinesin and muscle myosin. Reprinted by permission from J Howard, 'Molecular motors: structural adaptations to cellular functions', 1997 *Nature* **389** 561–7 (Macmillan).

Most use the gradient of hydrogen ions that is found across the membranes of essentially all living cells; biologists commonly call hydrogen ions *protons*, and this electrochemical gradient is termed the *protonmotive force*. In some bacteria, though, such as *Vibrio cholerae*, sodium ion flux is used to power the flagellar rotary motor [27]. A particularly interesting case of a rotary motor is the F1 ATPase used to synthesize ATP in mitochondria. This molecular motor (or dynamo) converts chemical energy in the form of a proton gradient into mechanical energy in the form of a unidirectional rotation and then back again into chemical energy in the form of ATP [28]. In the presence of excess ATP, the motor can be made to run backwards, with no apparent loss of efficiency, pumping protons against their electrochemical gradient at the expense of ATP hydrolysis.

Linear stepper motors are much more common in eukaryotic forms of motility. These motors move along preassembled linear tracks by coupling binding to the track with ATP hydrolysis and a large-scale protein conformational change. The first linear stepper motor to be purified and characterized was myosin, the motor that drives filament sliding in skeletal muscle contraction (see figure 3). The track for myosin is the actin filament, or microfilament, a helical polymer formed by noncovalent self-association of identical globular subunits. It is now clear that skeletal muscle myosin is just one member of a large family of related myosin motor proteins [29]. All use actin filaments as a track and all share a similar motor 'head' domain, but the 'tail' domain that attaches the motor to its cargo is highly divergent. Some tails cause dimerization, leading to the formation of a two-headed motor, as is the case for muscle myosin (myosin II) and several other classes. Currently, there are at least 18 different classes of myosins known, and each class may comprise dozens of different members even in a single organism. Various forms of myosin in humans are responsible for biological movements as diverse as muscle contraction, cell division, pigment granule transport in the skin, and sound adaptation in the hair cells of the inner ear. The exact mechanical features of each type of myosin motor head, including the stepping speed, stepping distance, fraction of time bound to the track versus free, and amount of force generated, appear to be carefully tuned to their biological functions [30]. Myosins of different classes have step sizes ranging from 5 to 36 nanometers and generate forces generally on the order of a few piconewtons, performing between 1 and 20 steps per second. In the case of myosin V, which is responsible for transport of pigment granules in mammalian skin cells, a single molecule of the motor protein may be sufficient for granule movement, while in skeletal muscle, nearly crystalline arrays of hundreds of myosin II molecules act in concert to drive the shortening of each sarcomere. A unique and speed-optimized plant myosin drives the extremely rapid circulation of chloroplasts in *Chara*, while another unusual myosin is associated with gliding movements of eukaryotic parasites. The mechanisms by which different myosins can exhibit such a wide variety of mechanochemical properties are not yet fully understood.

Similar functional tuning of motor mechanical properties is found in another abundant and diverse family of linear stepper motors, the kinesins, which use microtubules rather than actin filaments as a track [31]. Like the myosins, the kinesins are most similar in the motor head domain and show great divergence in the tail. Kinesins are involved in many types of intracellular transport, including transport of organelles down nerve axons and chromosome segregation. Although myosins and kinesins use different tracks and have different mechanisms for coupling the energy derived from ATP hydrolysis to the protein conformational change associated with force generation [30], recent work determining the atomic structure of canonical members of both families has revealed that the core protein structures are remarkably similar, an almost certain indication that myosins and kinesins are derived from the same common ancestor. The common motor core represents a fundamental physical unit coupling ATP hydrolysis to a small movement of two alpha helices in the protein structure; additional protein levers and latches convert this small molecular movement into the large and various functional steps of the motor proteins [32]. Another family of linear stepper motors that walks on microtubules, the dyneins, is less well-characterized. It is not yet known whether dyneins also share a common ancestor with kinesin and myosin or whether they might represent an independent, convergent development of stepper motor function. Dyneins also play a part in intracellular transport and chromosome segregation. The fastest and strongest dyneins are those responsible for the sliding and bending of the

microtubules inside eukaryotic flagella and cilia during cell swimming [6].

In addition to serving as the tracks for some kinds of linear stepper motors, microtubules and actin filaments can assemble and disassemble rapidly to change the shape of the cell and to produce force on their own [33, 34]. In these forms of biological force generation, the chemical energy comes from nonequilibrium protein polymerization, although ultimately the cellular pools of polymerizing actin and tubulin subunits are maintained in a steady state far from chemical equilibrium due to a coupling between protein polymerization and ATP or GTP (guanosine triphosphate) hydrolysis [35]. Force generated by actin polymerization is responsible for extension of the acrosome in some kinds of animal sperm [23], as well as the mating structure in *Chlamydomonas* [25]. With the filaments arranged in a slightly different geometry, actin polymerization is also the major driving force for cell protrusion at the leading edge in amoeboid motility [36] and for the movement of certain kinds of bacterial pathogens, including *Listeria monocytogenes* [37]. In an unusual form of amoeboid motility, the crawling sperm of nematodes (roundworms) uses an assembly and disassembly motor built by a unique protein, MSP (major sperm protein) [38]. The striking degree of resemblance between the crawling nematode sperm and a conventional crawling cell that uses actin assembly and disassembly for protrusion at the leading edge demonstrates that the physical rules governing the mechanics of cell motility are largely independent of the exact molecular identity of their components.

Whereas rotary motors and linear stepper motors have been characterized in great physical detail and force measurements have been performed on single molecules of each class (see next section), assembly and disassembly motors are poorly understood. Only a few measurements of the amount of force generated by single microtubules have yet been made [39], and there is to date no direct measurement of the force generated by polymerization of a single actin filament. The final two classes of biological motors, the extrusion motors and the prestressed springs, demand even more urgent attention. While the biological phenomena are well described and some initial physical and mathematical characterization has begun for each [9, 24], the physical models currently lack predictive detail.

Most interesting and complex forms of biological movement require the coordinated action of many kinds of molecular motors acting simultaneously. For example, the construction of the mitotic spindle and the process of chromosome segregation require forces generated by microtubule assembly and disassembly, as well as the action of about a dozen different kinesin and dynein motors. Similarly, amoeboid crawling motility requires actin filament assembly and disassembly, as well as at least three kinds of myosin motors. While reductionist techniques have enabled us to identify the molecules involved in many forms of biological movement, a complete understanding of the process demands a complementary synthetic approach to bring together biological characterization with physical measurements and mathematical modeling of properties emergent from large collections of motor elements.

4. Measurements of cell movements and forces

Identification of the molecular machineries that power cell motility has raised two major questions: (1) what is the mechanical behavior of the individual motor protein components and (2) how are proteins with diverse mechanical roles organized to produce coordinated mesoscopic and macroscopic movements? Answers to the first question require experimental methods capable of isolating the biophysical activity of individual or small groups of proteins, and answers to the second rely on mathematical models capable of predicting collective behavior from measured properties. The development of optical and force microscopy techniques over the last several decades has significantly advanced our quantitative understanding of cell motility at the level of single molecules, collections of molecules, and whole cells.

Time-lapse video microscopy produced early measurements of cell movements and internal dynamics [40]. Imaging methods based on polarization, phase, differential interference contrast, and dark-field microscopy can be used with conventional or high-speed strobe light illumination to capture motion of single flagella on sperm, migration of epithelial cells, and contraction of muscle cells. The development of fluorescent markers capable of localizing specific proteins in time and space has spawned powerful quantitative microscopy techniques. For example, a ratiometric version of fluorescence localization after photobleaching (FLAP) was recently used to show that monomeric actin delivery to the leading edge of motile cells exceeds the speed expected for simple diffusion [41]. At the single molecule level, centroid tracking of a fluorescent marker attached to one head of myosin V, a two-headed processive molecular motor that moves along actin filaments, has revealed that the motor steps are consistent with a hand-over-hand model for walking rather than an inchworm model in which one head always leads the other [42]. Optical microscopy, and in particular fluorescence microscopy techniques for labeling specific proteins, continues to be a basic tool for quantifying the spatial distribution and time variation of molecular components and cell morphology.

While position information guides the cell motility story line, mechanical information is required for a full Newtonian description of the process. Force measurements can be extracted from optical images by allowing motile cells or force-exerting molecules to interact with materials of known mechanical properties. A dramatic example from more than 20 years ago was the demonstration that fibroblasts cultured on a thin silicone rubber sheet would exert forces sufficient to wrinkle the substrate (see figure 4) [43]. Shear force estimates of 0.001 dynes per micron were made by inducing similar wrinkles with a calibrated glass microneedle. Glass microneedles with calibrated spring constants have been used in other experiments to directly measure forces, as in early work investigating muscle contraction [44]. Recently, glass microneedles and flexible substrates were

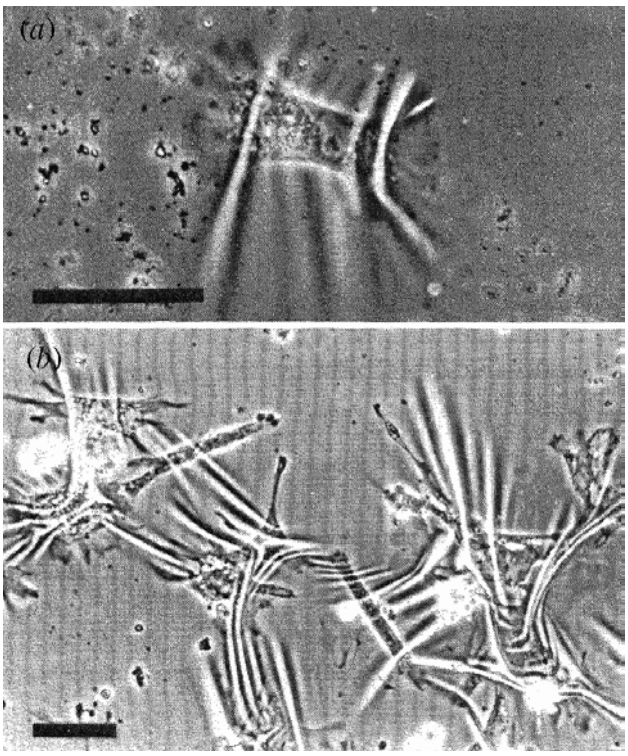

Figure 4. Chick heart fibroblast wrinkling a silicone rubber substrate. Scale bar in (*a*) is 50 μm and (*b*) is 100 μm. Reprinted with permission from A K Harris, P Wild, and D Stopak, 'Silicone rubber substrata: a new wrinkle in the study of cell locomotion', figure 2A, B. 1980 *Science* **208** 177–9.

used to demonstrate the role of tensile stress in microtubule growth and cell motility [45]. Other materials with known mechanical properties can be used to infer force information. For example, estimates of actin polymerization forces on artificial cargo have been obtained from deformation of lipid vesicles [46, 47].

Investigations of the mechanics of single molecules in vitro have motivated the development of optical, magnetic, and atomic force microscopy methods for probing biological forces ranging from 10^{-12} to 10^{-6} newtons [48]. Optical traps or tweezers use focused light to elastically confine a bead near the focus, with trap stiffness depending on optical power. Displacements of a bead linked to a motor protein or other force-generating molecule are converted into force measurements and have been used to observe single steps of the linear stepper motors kinesin [49] and myosin (see figure 5) [50]. In magnetic tweezers, a magnetic particle is manipulated by an external magnetic field to generate force. Superparamagnetic beads attached to a macrophage were used to guide cellular extensions with a force as small as 0.5 nanonewtons [51]. Atomic force microscopy (AFM) has also been used to investigate the mechanical properties of motile cells. Atomic force microscopy is based on the elastic deflection of micromachined cantilevers to measure and apply forces. Originally developed as a surface imaging tool [52], AFM has found applications in biology such as unfolding single proteins [53] and measuring whole-cell mechanics [54]. Maps of a cell's elastic modulus can be obtained by applying a known force and measuring actual deflection, a technique known as *force mapping*, which has been applied to quantify mechanical differences in the active and stable edges of motile fibroblasts [55]. On their own, the physical properties revealed by a wide range of measurement techniques provide a glimpse of the mechanical diversity of the single molecules and whole cells. Together, these properties provide inputs for mathematical models designed to capture the macroscopic behavior of cell motility.

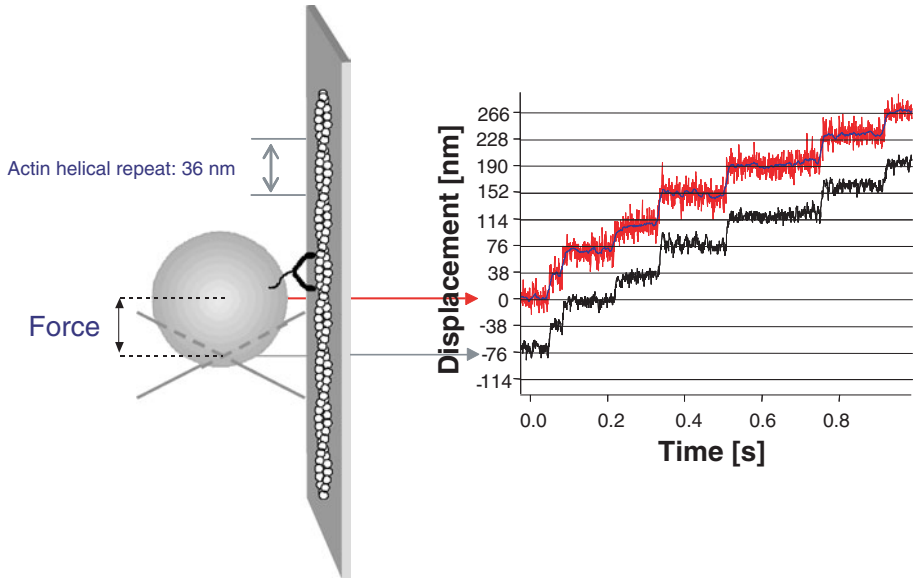

Figure 5. Single-molecule stepping of myosin V measured with optical tweezers. Schematic diagram and data courtesy of Jim Spudich, Stanford.

5. Contributions of physical modeling

A complete understanding of cell motility, involving the coordinated activities of thousands of proteins and signaling molecules, is impossible to capture in a single force measurement or mechanical property. Mathematical modeling provides a link between the observed behavior of cells (speed, shape change) and the properties of single molecules or modules of constituent proteins. The reductionist strategy of determining the identity and rate constants of, for example, the key molecular players in lamellipodial extension has been largely successful [56], but modeling must rebuild the process and verify our understanding with predictive power. Current models can be divided into two categories based on the level of approximation: (1) molecular and (2) nonmolecular or continuum models. Molecular or stochastic models attempt to capture the effect of Brownian motion on the activity of individual proteins and provide a mechanistic explanation of protein behavior. In contrast, nonmolecular or continuum models of cell motility describe movements in terms of bulk or averaged material properties of the cell and its environment. We give a few examples of both types of models as they have been applied to cell motility associated with actin polymerization.

In the case of *Listeria monocytogenes*, a bacterial pathogen that harnesses the assembly and disassembly motor of actin polymerization to power its intracellular and intercellular movement, molecular and nonmolecular models have been proposed to describe its motion. The Brownian ratchet models for actin polymerization [57], like those of molecular motor movement [58], relate movement of the bacterium to stochastic fluctuations in the filaments as a function of external load. Inclusion of elastic filament bending and filament attachment properties into the brownian ratchet model [59] yielded a force-velocity curve that shows an exponential decay and is consistent with some observations of *Listeria* motion [60].

Continuum models ignore local molecular properties in favor of bulk mechanical properties. In a model by Gerbal *et al* [61], *Listeria* motility is described by elastic energy storage in a compressible gel. Bulk mechanical properties of the gel substitute for the complex network of actin filaments and accessory proteins responsible for bacterial movement and simplify analysis. Although gel properties used in the model do not account for molecular kinetics, it is consistent with experiments showing 'hopping' behavior of *Listeria* under some conditions [62].

Other continuum models have focused on descriptions of the bulk properties of static cells. While early descriptions of the cell as a bag of viscoelastic material have been abandoned, recent models have succeeded in identifying several important material properties of various cells grown under laboratory conditions. The tensegrity analogy for cell structure proposes that the cell can be described as an interconnected array of tension and compression elements, like the architectural tensegrity constructs of Buckminster Fuller [63]. Investigations of prestressed cells using traction-force microscopy have shown a linear increase in shear modulus, consistent with some forms of a tensegrity model [64]. In contrast, microrheological measurements of smooth muscle cells using a magnetic bead assay have shown that the cells are well-described by a frequency scaling law over at least five orders of magnitude, suggesting that bulk properties of the cell more closely resemble soft glassy materials near a phase transition than gels [65]. Simplifications offered by continuum models help to make complex mechanical processes tractable, with fewer and often more easily measured variables, and models that accurately predict cell behavior then pose the question: how is the continuum description explained in molecular terms?

One of the most important features of cell motility models, whether molecular or nonmolecular, is that they make testable, quantitative predictions that allow specific mechanisms to be ruled in or out by subsequent experimental investigation. For example, the original Brownian ratchet model of actin motility was based on diffusion of the cargo and consequently predicted that larger cargo should move more slowly [57]. Experiments in reconstituted systems with polystyrene beads over a range of sizes did not show the expected size dependence, indicating that other factors must dominate and leading to models with improved predictive power [59]. Predictions from recent models of actin-based motility now await testing. Branching structure models of actin polymerization based on autocatalytic and nucleation branching structures predict differences in actin filament density and force-velocity curves, depending on the exact nature of the biochemical interactions among neighboring actin filaments [66, 67]. A model of actin polymerization in the leading edge predicts a relationship between the number of active filaments and the membrane velocity with a local maximum [68]. With improvements in optical and force microscopy techniques, repeated rounds of collaboration between experimental measurement and theoretical modeling will approach descriptions of cell motility that capture macroscopic behavior and microscopic detail.

6. Challenges to understanding the physics of cell motility

Biological cells—inhomogeneous, dynamic, and complex—are unlike any other material familiar to most physical scientists. The challenge of developing a complete physical description of how and why cells move has opened a wide range of experimental and modeling opportunities and given rise to numerous collaborative investigations. The identity and role of many parts of the motility machinery remain to be found, particularly those involved in control and regulation. New ways of describing material properties of cells that capture the temporal changes and environmental stimulus are needed. New experimental techniques are required to extract physical information from motile cells, and new models are needed to link molecular- and nonmolecular-scale models that can predict new targets for measurement. Fruitful investigation in cell motility at the interface of biology and physics will demand an increase in the number of scientists fluent in cross-disciplinary languages, biologists who can frame

questions in terms of Monte Carlo simulations and piconewton force measurements, as well as physicists comfortable with genetic analysis and protein purification. In addition to explaining fundamental biological processes that are necessary for survival, investigation of the mechanics of cell motility promises to exemplify interesting dynamic physical properties of living materials and provide tools for nanotechnology and bioengineering.

Glossary

Cytoskeleton. Integrated system of intracellular filamentous protein polymers (including actin filaments and microtubules) and their ancillary associated proteins (including myosin and kinesin) that contribute to cell structure, organization, and motility.

Flagella. In prokaryotes, rigid helical filaments driven by rotary motors; in eukaryotes, bundles of microtubules that use dynein motors to produce a wave-like motion (structurally and functionally related to cilia).

Motility. Directed motion driven by energy-consuming motors (contrasted to *mobility*—passive motion driven by thermal or other external mechanisms).

Molecular motor. Biological mechanism that converts chemical energy into mechanical energy, used by a cell to generate directed motion. A single motor can be as simple as a single polypeptide chain or as complicated as a giant macromolecular complex with hundreds of protein and proteoglycan constituents. Chemical energy used by molecular motors is generally either in the form of a high-energy chemical bond (as in ATP or GTP) or an ion gradient across a membrane.

Sessile. Nonmotile, as a plant (used to describe cells or organisms)

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Further reading

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Bray D 2001 *Cell Movements: From Molecules to Motility* 2nd edn (New York: Garland)
Howard J 2001 *Mechanics of Motor Proteins and the Cytoskeleton* (Sunderland, MA: Sinauer Associates)
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