The cytoskeleton, cellular motility and the reductionist agenda

Thomas D. Pollard

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven Connecticut 06520-8103, USA
(e-mail: thomas.pollard@yale.edu)

Eukaryotic cells depend on cytoskeletal polymers and molecular motors to establish their asymmetrical shapes, to transport intracellular constituents and to drive their motility. Cell biologists are using diverse experimental approaches to understand the molecular basis of cellular movements and to explain why defects in the component proteins cause disease. Much of the molecular machinery for motility evolved in early eukaryotes, so a limited set of general principles can explain the motility of most cells.

Three cytoskeletal polymers — actin filaments, microtubules and intermediate filaments (Table 1) — cooperate to maintain the physical integrity of eukaryotic cells and, together with molecular motors, allow cells to move themselves and their intracellular components. Although cellular motility has fascinated small groups of biologists for 300 years, interest in these processes has now spread to biologists more generally. The field has expanded as a result of insights gleaned about molecular mechanisms and the participation of cytoskeletal and motility molecules in many aspects of cellular function, including embryology, learning and memory, spread of cancer and microbial pathogenesis. The carefully regulated assembly of the cytoskeletal polymers and action of the associated motors is largely responsible for establishing cellular architecture and thus tissue structure.

This collection of reviews will bring readers up to date on several active areas of research. Howard and Hyman (page 753) explain how assembly and disassembly of microtubules produce forces to transport some intracellular molecules, chromosomes and organelles. Cellular locomotion powered by the assembly and disassembly of actin filaments[1] has many parallels with these microtubular mechanisms. Schliwa and Woehlke (page 759) cover the molecular motors that interact with actin filaments and microtubules to generate tension in the cytoskeleton as well as to move cargo as large as nuclei and as small as RNA molecules. Nelson (page 766) reviews how cells use cytoskeletal polymers and motors to generate asymmetry. Gruenheid and Finlay (page 775) cover the many ways that infectious organisms can hijack the motility system for their own purposes, while Scholey *et al.* (page 746) describe what we know about the segregation of chromosomes during mitosis and pinching daughter cells in two during cytokinesis.

These are spectacular examples of events where the cytoskeletal polymers and motors transiently assemble complex machines to carry out vital processes with high fidelity. The machines used for cellular locomotion, intracellular transport, mitosis and cytokinesis consist of millions of protein molecules held together by relatively weak, non-covalent bonds, which allows these machines to disassemble when their jobs are done, recycling their protein components for use at a later time.

In keeping with their fundamental contributions to cellular integrity and function, defects resulting from mutations in the genes for cytoskeletal and motility proteins cause human disease. Recent examples include mutations in ankyrin (part of the membrane skeleton), which cause one type of cardiac arrhythmia[2], in titin in cardiomyopathies[3], and in myosin-II in congenital defects of the brain and kidney[4].

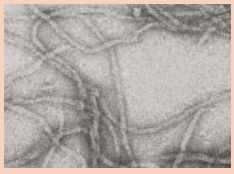
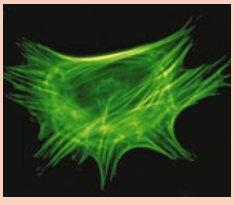
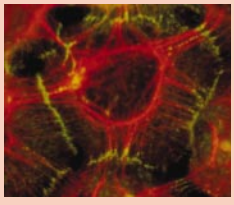
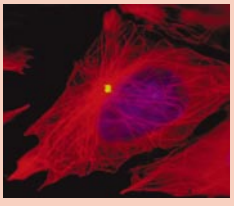
This perspective illustrates the power of the reductionist approach in cell biology and in studying the molecular basis of cellular movements in particular. Implementation of this agenda is based on three 'articles of faith'. First, owing to evolution from common ancestors, modern cells use a common set of molecular mechanisms to carry out their basic functions. Consequently, cell biologists believe that analysis of any experimentally tractable organism provides insights about general principles that will apply to most cells. Second, knowledge of the structures and functions of the individual parts of molecular machines reveals much about the workings of ensembles of molecules. And, third, a critical test for understanding is reconstitution of a complex process from purified components in 'wet' biochemical experiments and/or in computer simulations. Here I consider where the field stands with respect to these underlying beliefs and I conclude with a brief review of actin-based cellular motility, a topic not covered by the authors of the accompanying reviews.

Evolution

All five articles emphasize the contrast between the vast diversity of cellular behaviours and the unity of the underlying molecular mechanisms. Animal, plant and fungal cells differ remarkably in size, shape, motility and associations with other cells. Tiny yeast cells and most plant cells are trapped inside a cell wall, whereas animal cells can be either motile or confined to tissues by interactions with their neighbours. Most yeast segregate their chromosomes with a mitotic apparatus confined to the nucleus, whereas animals and plants have cytoplasmic mitotic apparatuses. Plants seem to rely largely on creation of a new plasma membrane and cell wall for cytokinesis, while both fungi and animals use a contractile ring of actin and myosin to divide.

Despite this diversity at the cellular level, the underlying mechanistic unity is now clear at the molecular level. All eukaryotic cells, in spite of their superficial differences, have inherited 'core mechanisms' (to quote Nelson) that are responsible for their structure and motility, including mitosis and cytokinesis. This core machinery appeared in a highly refined and effective form very early in the evolution of eukaryotes. Cells lacking this core machinery were lost. Given hundreds of millions of years since the main groups of

Table 1 **Eukaryotic cytoskeletal polymers**

Polymer	Actin filament	Microtubule	Intermediate filament
Protein subunit	Actin monomer	Tubulin heterodimer	Various proteins with an α-helical coiled-coil
Evolutionary origins	Prokaryotic hexokinase → prokaryotic actin-like proteins	Prokaryotic FtsZ	Early eukaryotic nuclear lamins
Polymerization by nucleation/elongation	Yes	Yes	Probably
Bound nucleotide	ATP	GTP	None
Ageing by nucleotide hydrolysis and phosphate release	Yes, allows binding of proteins that promote disassembly	Yes, destabilizes polymer	No
Flux of subunits through polymer at steady state (treadmilling)	Yes, very slow	Yes, slow	No
Dynamic instability (spontaneous fluctuations in length at steady state)	No	Yes, dramatic	No
Track for motors	Yes, 20 families of myosins	Yes, several dyneins and many families of kinesins	No
Electron micrographs of polymers			
Fluorescence micrographs of cells with polymers			

Micrographs reproduced with permission from ref. 31. The left fluorescence micrograph is from I. Herman, Tufts Medical School; the middle is from E. Smith and E. Fuchs, University of Chicago; and the right is from G. Borisy, University of Wisconsin.

eukaryotes separated from each other and given different selective pressures, their genomes have diverged significantly. A few genes for this core machinery were lost in specialized cells. Other genes acquired mutations that increased fitness for their organism's lifestyle. Some genes duplicated and then diverged to provide specialized functions. Although evolution refined the ancient mechanisms in each species, the core strategies are still used in contemporary cells that bear little superficial resemblance to each other. This allows investigators to search for general principles in those organisms that are most tractable for experimentation. Far from being an impediment, the diversity at the species level allows cell biologists to view the fundamental mechanisms of motility from a variety of perspectives.

Genes for actin and tubulin arose in prokaryotes[5]. Although the primary structures diverged extensively, crystal structures of prokaryotic actin-like and tubulin-like proteins are remarkably similar to their eukaryotic counterparts. Bacterial FtsZ binds GTP just like tubulin but polymerizes into long ribbons that participate in cytokinesis. Eukaryotic tubulin is a heterodimer of similar α- and β-subunits that assemble into cylindrical polymers (Table 1). The GTP bound to tubulin is hydrolysed and the γ-phosphate dissociates soon after incorporation of each tubulin molecule in a polymer. Dissociation of the γ-phosphate puts tubulin into a strained conformation that favours disassembly of the microtubules (see review by Howard and Hyman, page 753). Bacterial MreB binds ATP and forms actin-like filaments[5] that are required for the elongated shape of rod-like bacteria. Some bacterial actins also help to partition DNA during mitosis[6]. (The assembly properties of actin are considered below.) In a fascinating role reversal early in eukaryotic evolution, actin filaments took over cytokinesis and microtubules assumed the partitioning of the genome.

Although actin filaments and microtubules differ in origin and structure, their shared features (Table 1) shows that evolution favoured extensive convergence of function. Moreover, nematodes evolved completely different cytoskeletal polymers for their amoeboid sperm. Polymers of 'major sperm protein' lack any molecular similarity to actin, but carry out a cycle of assembly and disassembly that mimics that of actin in motile cells[7].

Intermediate filaments arose during eukaryotic evolution rather than in prokaryotes and share little with the other cytoskeletal polymers. The rod-shaped protein subunits of intermediate filaments consist of a coiled-coil of α-helices and do not bind nucleotides. Owing to the symmetry of the subunits, the polymers are not polar like actin filaments and microtubules. Duplication and divergence of the genes for intermediate filament proteins produced a family of related genes in vertebrates. The protein products are expressed selectively in specialized cell types where they act as intracellular tendons that resist deformation of cells and tissues. Hair is composed of keratin intermediate filaments and illustrates the mechanical properties of these polymers. Mutations that interfere with the assembly of intermediate filaments result in mechanical fragility of the cells and tissues that depend upon them for their integrity. One example is mutations that compromise the keratin intermediate filaments in skin, cause blistering diseases[8].

The molecular motors that move along microtubules and actin filaments had two origins. Dyneins are part of the family of AAA ATPases[9] that also contribute to protein folding (Hsp100 chaperones), membrane traffic (*N*-ethylmaleimide-sensitive factor or NSF) and DNA synthesis (clamp loader proteins). The kinesin and myosin families of ATPase motors share a common core structure and may have the same common ancestor as the GTPases involved in signalling and protein synthesis[10]. Although GTPases are present in prokaryotes, compelling evidence for prokaryotic motors is still lacking.

The reductionist approach

Our understanding of the cytoskeleton and cellular motility is a triumph of the reductionist strategy, the approach that now dominates

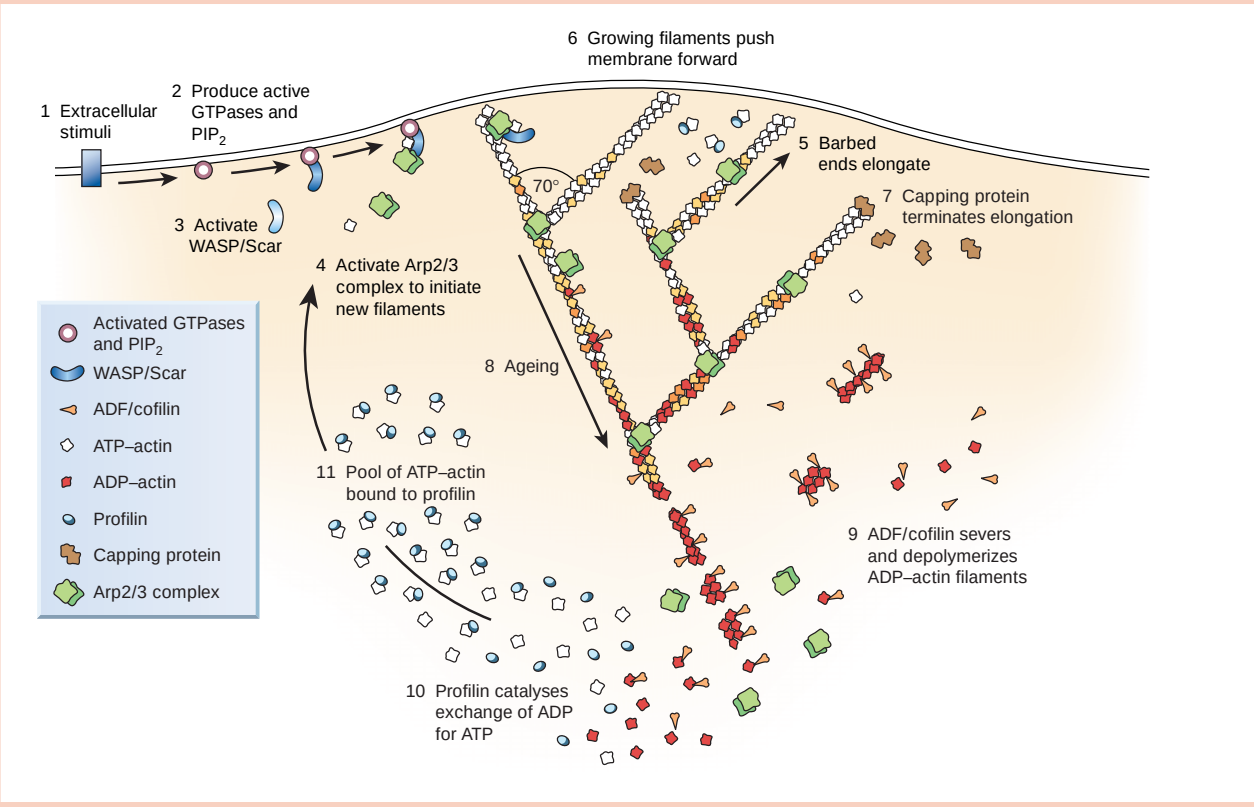


Figure 1 The dendritic-nucleation model for protrusion of lamellipodia. External cues (step 1) activate signalling pathways that lead to GTPases (2). These then activate Wiskott–Aldrich syndrome protein (WASP) and related proteins (3), which in turn activate Arp2/3 complex. Arp2/3 complex initiates a new filament as a branch on the side of an existing filament (4). Each new filament grows rapidly (5), fed by a high concentration of profilin-bound actin stored in the cytoplasm, and this pushes the plasma membrane forward (6). Capping protein binds to the growing ends, terminating elongation (7). Actin-depolymerizing factor (ADF)/cofilin then severs and depolymerizes the ADP filaments, mainly in the 'older regions of the filaments (8, 9). Profilin re-enters the cycle at this point, promoting dissociation of ADP and binding of ATP to dissociated subunits (10). ATP–actin binds to profilin, refilling the pool of subunits available for assembly (11). (Image based on an original figure from ref. 32.)

research in cell biology. Sophisticated methods drive rapid progress, but we should aware of the limitations of these methods and the unfulfilled items on the reductionist agenda. The reductionist tasks include an inventory of the relevant molecules, determination of molecular structures, identification of molecular partners, measurement of rate and equilibrium constants for each reaction, localization of the molecules in live cells, physiological tests for participation in cellular processes and formulation of mathematical models to understand the system's behaviour. Each review in this Insight section emphasizes parts of this agenda.

Reductionism starts with a list of the components. Most of the cytoskeletal proteins were discovered the 'old-fashioned' way, using purification by biochemical fractionation. Complete genome sequences and expressed sequence tag collections have expanded the inventory of cytoskeletal and motor proteins, particularly the diversity of isoforms of many of the proteins found in higher organisms. In a few cases experts have completed the annotation of selected genomes and defined the size of certain gene families such as myosins, which consists of more than 40 genes in humans[11]. Similar work remains to be done for many other cytoskeletal gene families. Far less is known about the diversity of products generated by alternative splicing of pre-messenger RNAs.

Genetic screens and yeast two-hybrid assays have accelerated detection of protein partners, but traditional biochemical assays and affinity chromatography remain useful, particularly when empowered by sensitive analytical methods such as mass spectrometry. When scaled up to sample entire genomes or proteomes, these assays produce impressive interaction maps[12,13]. Such efforts have saved an immense amount of work and laid out a broad research agenda that is required to understand each interaction. These maps are, of course, a beginning rather than an end, as simple knowledge of an interaction will not explain how anything actually works.

Structure determines function, so the field eagerly awaits each new structure. Recent crystal structures include tubulin bound to a small regulator protein Op18/stathmin (see review in this issue by Howard and Hyman, page 753), bacterial actin and tubulin homologues[5], and Arp2/3 complex (a seven-subunit nucleator of actin filaments[14]). Lacking crystals, three alternative approaches have yielded valuable structural information. First, Wiskott–Aldrich syndrome protein (WASP), a multi-domain protein that activates Arp2/3 complex, has been studied one domain at a time by nuclear magnetic resonance[15,16]. Second, homology modelling based on other AAA ATPases was used to construct a preliminary model of dynein[9]. And third, technical advances in processing electron micrographs yielded an 8-Å structure of the microtubule[17]. Electron microscopy of single dynein molecules has recently led to a proposal for the mechanism of their ATP-driven power stroke[18]. Much work remains to complete a reference set of structures of cytoskeletal proteins.

Tracking the suspects

Light microscopy of live cells containing proteins tagged with fluorescent markers has revolutionized much of cell biology and replaced fluorescent antibody methods for many purposes. Expression of proteins fused to green fluorescent protein (GFP; and related proteins with different spectral properties) has made it possible to localize and study the dynamics of virtually any protein inside a living cell (and

even in tissues of live organisms; see review by Howard and Hyman, page 753, for examples). Investigators have embraced these methods with justifiable enthusiasm, but caution is required, as some fusion proteins cannot take the place of their wild-type counterparts in gene replacement experiments. Genetic manipulations make such controls routine in yeast laboratories, but they are rarely done in experiments on animal or plant cells.

Speckle microscopy has increased the power of fluorescent protein methods[19]. Expression of a low level of a GFP fusion protein or microinjection a low concentration of purified protein labelled with a fluorescent dye leads to stochastic incorporation of labelled protein into microtubules, actin filaments or other cellular structures. The resulting speckles of fluorescence serve as fiduciary marks for orientation as the labelled structures move or turn over in live cells (see, for example, ref. 20).

Single-particle assays continue to make valuable contributions to understanding motility. One example is provided by the surprising solution to decades of controversy surrounding the mechanism of slow axonal transport. In this process, proteins such as the subunits of intermediate filaments move slowly (only 1–100 nm per second) from their site of synthesis in a neuronal cell body to the end of an axon or dendrite. Different experimental approaches gave apparently conflicting results regarding the movement of the molecules, whereas observation of single intermediate filaments revealed that they actually move rapidly but infrequently[21]. Propelled by motors, they move in fits and starts (but mostly stops) along microtubules.

Another example is bacteria that usurp the cytoplasmic actin system for propulsion through the cytoplasm of host cells. Observations of single bacteria and particles coated with bacterial proteins (or other activators) have defined the physics of the process[22] and allowed reconstitution of the machinery from pure proteins[23]. Similarly, much has been learned about the behaviour of microtubules[24] and actin filaments[25] by real-time observations of single polymers.

Knock downs and knock outs

Depletion of a protein from a cell remains the standard to assign function at the cellular level. Many laboratories continue these experiments one gene at time using gene deletion in genetically tractable organisms. A complete set of deletion mutants for the budding yeast *Saccharomyces cerevisiae* has accelerated phenotyping. Depletion of mRNA and protein by RNA interference is faster, applicable to a growing range of cells and amenable to scaling up to screen the entire proteome for participation in a process such as cytokinesis (see review by Scholey *et al.*, page 746). However, in depletion experiments (as opposed to deletion experiments) one must keep in mind that severe reductions in concentration (or losses of affinity) may be required for physiological defects to appear; so false negatives are likely.

A complementary approach widely used in drug development and in a few academic laboratories is to screen target molecules or target cellular processes for inhibition with a library of small chemical compounds (for example, monasterol[26]). 'Chemical genetics' or 'chemical genomics' are neologisms for the broadened scope of this traditional pharmacological approach. Given a library of sufficient size and diversity, it seems possible to find an inhibitor for most proteins. If specificity can be established, small-molecule inhibitors have exceptional value in analysing cellular processes, particularly if inhibition is reversible on a biologically relevant timescale of seconds to minutes.

Reaction mechanisms and systems properties

With some exceptions, the definition of reaction mechanisms still lags is most parts of this field. Chemical kinetics and measurements of force and motion of single molecules have established the mechanisms of several kinesins and myosins (see ref. 10, and review in this issue by Schliwa and Woehlke, page 759). This work is essential, because history has revealed repeatedly that mechanisms remain a matter of speculation until cellular concentrations, affinities and reaction rates are known. Genetic interactions and identification of partners by semi- (or un-)quantitative precipitation assays are essential to initiate an investigation of mechanisms, but in every case known to me, the mechanism has turned out to be too complicated to understand without information about rates. Complete mechanisms are inevitably more interesting and pregnant with biological implications than superficial explanations.

Any cellular process involving more than a few types of molecules is too complicated to understand without a mathematical model to expose assumptions and to frame the reactions in a rigorous fashion. Second- and third-generation mathematical models are now being used to guide thinking and experimentation on the mechanisms of bacterial chemotaxis[27] and of the yeast cell cycle[28]. The most advanced mathematical models in the field of cell motility deal with the actin filaments at the leading edge of continuously moving cells.

Cellular locomotion based on actin assembly

Primitive eukaryotes developed a mechanism to move towards food and away from harm that is based on the assembly of actin filaments (Fig. 1), which push the cell forward as the polymers grow at the leading edge of the cell (reviewed by ref. 1). All contemporary eukaryotes seem to use some variation of this ancient mechanism, although its manifestations vary from the movement of small 'patches' of actin filaments associated with the cell membranes of fungi to the rapid locomotion of cells such as human leukocytes. Genes required for this mechanism are found in protozoa, fungi, plants and animals. Although these genes are ancient, they have been conserved well enough through evolution that the protein parts seem to be fully interchangeable across species in biochemical assays.

Analysis of actin-based cellular motility illustrates how the reductionist strategy can be used to decipher a complex mechanism. So far, many of the key proteins have been identified and shown to reconstitute motility in a model system[23], all of their atomic structures are known, most of the rate and equilibrium constants have been measured, electron microscopy has revealed the organization of the machine in cells and a mathematical model correctly predicts the rate of movement[29].

Like tubulin, actin binds a nucleoside triphosphate, in this case ATP. After an actin molecule incorporates into a filament, the γ-phosphate is hydrolysed rapidly from the bound ATP. Dissociation of the γ-phosphate is slow, and ADP–actin has a lower affinity for the end of the filament, promoting dissociation and depolymerization.

This actin polymerization machine is intrinsically quiescent, but can be turned on by attractive chemical signals that direct cells such as protozoa, white blood cells or fibroblasts towards nutrients, prey or a tissue home. Acting through a variety of receptors, these cues activate signalling pathways that lead to small proteins that bind and hydrolyse GTP. These GTPases then activate proteins related to the product of the gene mutated in a human immunodeficiency disease called Wiskott–Aldrich syndrome. WASP and related proteins activate a large assembly of seven proteins called Arp2/3 complex, including two actin-related proteins (Arp2 and Arp3). Arp2/3 complex initiates a new filament as a branch on the side of an existing filament. Each new filament grows rapidly, fed by a high concentration of actin stored in the cytoplasm bound to the small protein profilin. Growth of the filaments pushes the plasma membrane (and the cell) forward. The energy comes from high-affinity binding of ATP–actin to the ends of filaments, similar to growing microtubules transporting cargo at their tips (discussed by Howard and Hyman, page 753). Initiation of new filaments as branches from the existing network provides a scaffold to push against.

The system is set up to terminate the growth of the filaments automatically before they grow so long that they do not push effectively and then to disassemble the network, so that the components can be recycled for an subsequent round of polymerization. First, capping protein binds to the growing ends, terminating elongation. Next, a

small protein called actin-depolymerizing factor (ADF)/cofilin binds weakly to the side of ADP–Pi actin filaments and promotes dissociation of the γ-phosphate. The ADP filaments become a target for higher-affinity binding of ADF/cofilin, leading to their severing and depolymerization. Profilin re-enters the cycle at this point, promoting dissociation of ADP and binding of ATP to dissociated subunits. ATP–actin binds to profilin, refilling the pool of subunits available for assembly.

Although many details of this mechanism remain unclear, a mathematical model incorporating both the molecular reactions and physical forces[29] correctly predicts the steady-state rate of cellular locomotion. This system has several advantages for modelling. It runs at steady state, the inventory of core proteins is small, the structures and concentrations of these proteins are known and biophysicists have measured many of the rate and equilibrium constants for the reactions. These models identify the variables that limit the rate of movement, such as the concentration of actin bound to profilin. In fact, when the concentration of unpolymerized actin is acutely lowered by releasing an actin-monomer sequestering protein locally in the cytoplasm, that part of a cell stops moving[30]. The models raise a number of questions that can be addressed by further experimentation. Is the concentration of unpolymerized actin bound to profilin really the parameter limiting the rate of movement? Do interactions of the growing filaments with the inner surface of the membrane inhibit capping, thus biasing growth in the forward direction? How is the network of short, branched filaments remodelled into a network long unbranched filaments deeper in the cytoplasm?

Unmet challenges

Although we now have in hand a broad outline of the strategies that evolution has provided cells to produce motility and asymmetry, actual understanding of the physical mechanisms will require completion of the reductionist agenda. We still have gaps in our parts list and especially in biochemical mechanisms. As this agenda nears completion, the shear complexity of most of the mechanisms driving cellular motility will force cell biologists to depend increasingly on mathematical models to test their hypotheses. Iterative cycles of quantitative modelling and quantitative experimentation are the only way to eliminate false but attractive hypotheses and to expose the valid features of models to rigorous scrutiny. Although rare in cell biology, this interplay of experiment and theory will gain in importance as the characterization of other systems advances. □

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