

Active Biological Materials

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Abstract

Cells make use of dynamic internal structures to control shape and create movement. By consuming energy to assemble into highly organized systems of interacting parts, these structures can generate force and resist compression, as well as adaptively change in response to their environment. Recent progress in reconstituting cytoskeletal structures in vitro has provided an opportunity to characterize the mechanics and dynamics of filament networks formed from purified proteins. Results indicate that a complex interplay between length scales and timescales underlies the mechanical responses of these systems and that energy consumption, as manifested in molecular motor activity and cytoskeletal filament growth, can drive transitions between distinct material states. This review discusses the basic characteristics of these active biological materials that set them apart from conventional materials and that create a rich array of unique behaviors.

1. INTRODUCTION

Cells exhibit an astonishing range of movements and shape changes (1). As evidenced by the swimming of *Spiroplasma*, the gliding of *Myxococcus*, and the crawling of macrophages, nature has produced molecular systems that interact physically with each other and their environment in complex ways. For each of these movements, small forces and rapid motions at the molecular scale must be coordinated in space and time to produce large forces and persistent motion at the cellular scale. Cells and the molecules that compose them must also adapt rapidly to changes in their surroundings to effectively search for food, escape harm, and defend against invasion. The mechanics and dynamics of the internal structures that drive cell movements are often thought of in terms of material properties such as elasticity and viscosity in an effort to develop mechanistic models of cell movements (2, 3). Yet the analogy between conventional materials of everyday experience and the active biological materials at work in cells challenges our notion of what constitutes a material and which properties of biological structures should be used to describe the material. At the same time, this analogy focuses our attention on the complex mix of length scales, timescales, and force scales that must be understood to explain the unique physical behaviors of cells.

In some ways, the study of active biological materials—the systems of molecules that drive cellular organization, shape change, and movement—is an old topic. Beginning centuries ago with van Leeuwenhoek's observations of single-celled "animalcules" swimming in drops of pond water, our understanding of cell movements has advanced dramatically through a combination of studies linking molecular activity to cellular behavior. A prime example is the case of muscle fibers. After early light microscopy techniques identified a structural regularity to muscle tissue in the late 1800s, biochemical purification methods succeeded in isolating and identifying two key molecules as myosin and actin in the 1940s (4). Around the same time, electron microscopy studies of thin section muscle samples revealed an arrangement of overlapping myosin thick filaments and actin thin filaments, and the sliding filament hypothesis was proposed. Through further biochemical characterization, and, critically, the demonstration that heavy meromyosin binds to actin filaments (5) and the reconstitution of filament sliding *in vitro* (6), the essential basis of the cross-bridge model of muscle contraction was established (7, 8). Before this level of detail was known, one could have treated muscle fibers as a conventional material with an elastic modulus and viscous creep. However, this description would miss the rich behavior of muscles that can be understood only once the organization and activity of the cell's cytoskeleton—the system of filaments, motors, and accessory proteins that define its structure—are known.

Today, all eukaryotic cells are known to contain a cytoskeleton, comprising a network of dynamic filamentous protein polymers that collaborate with a diverse set of binding proteins and molecular motors to form nature's most remarkable active material. Even bacteria, thought to have no cytoskeleton at the turn of the millennium, are now known to contain a diverse set of proteins capable of forming structural filaments (9). Yet even full knowledge of the parts of a system does not reveal its behavior any more than the parts of a disassembled watch offer clear insight into the mechanism of keeping time. It is critical to know how the different parts are organized in space and time. Despite great progress in the study of biological movements such as muscle contraction, our understanding of how the cytoskeleton of cells drives internal rearrangements and external shape changes is far from complete. The combination of two approaches, reductionist biochemical techniques used to identify specific molecules of importance and functional experimental techniques used to localize and characterize the behavior of those molecules within cells, has left a hole in our understanding at the level of mesoscopic systems of molecules. We may know which proteins are required for a process and how they are arranged in a cell, but we do not know how those proteins became arranged into that particular configuration nor how that configuration gives rise to the physical behavior under study. It thus remains unclear how the cytoskeleton

drives complicated movements such as those involved in endocytosis, phagocytosis, and crawling motility. The challenge now lies not in identifying the molecular components or their individual function, but rather in integrating the parts and their biochemical, mechanical, and energetic behaviors into a comprehensive understanding of cell movements and shape changes.

In recent years, the capability to study active biological materials outside of cells has emerged. When the proteins involved in a particular structure have been identified and purified in an active form, and once the conditions that control assembly have been discovered, the structure can be reconstituted *in vitro* to study its mechanics and dynamics in detail. With precise control over protein concentration, *in vitro* reconstitution can test the ability to generate a functional readout such as spatial organization or force. Importantly, this approach opens the door to direct comparisons between experimental data and detailed simulations that include all molecular players, something that cannot be done with whole cells. In this sense, the study of active biological materials is a young field, one that builds on the centuries of work before it but is only now tackling the question of how coordinated movement can emerge from a small variety of molecules. Recent research has begun to show how the interplay among length scales, force scales, and timescales makes active biological materials much richer in behavior than traditional materials that happen to comprise biomolecules. Here, we discuss experimental and theoretical perspectives on the cytoskeleton in the context of specific biological structures, focusing on its assembly, mechanics, and dynamics. Our intent is not to provide an extensive review of the literature but rather to call attention to basic concepts that help frame key questions facing the field.

2. ACTIVE BIOLOGICAL MATERIALS VERSUS STATIC CONVENTIONAL MATERIALS

The relevant properties of a material depend on its use. For a conventional material such as rubber in an automobile tire, relevant properties are macroscopic and passive, such as bulk elasticity. The rubber is designed to be stiff enough to resist significant extension by the load and to be sufficiently dissipative to damp transmission of vibrations. For an active biological material such as the actin cytoskeleton in a crawling cell, relevant properties are instead subcellular and dynamic, involving consumption of chemical energy to drive network assembly and coordinate movement. But the properties of biological materials are not limited to the scale of cells. In other biological contexts, the cytoskeleton can generate force on macroscopic length scales, as in the case of muscle fiber contraction, as well as on microscopic length scales, as in the case of filopodial protrusions of neuronal growth cones. The consumption of energy and the lack of a clear separation in length scales and timescales are key differences from conventional materials that impact the measurement, analysis, and interpretation of active biological materials.

A conventional material is typically placid and uniform on the macroscopic scale of the sample. Its physical properties may be strongly influenced by transient fluctuations, but their correlations typically decay over microscopic lengths (~ 1 nm) and persist for only very short times (~ 1 ns). As a result of these well-separated scales, it is possible to describe and model material functions at a continuum level, without reference to the underlying molecular origin of structure and dynamics. For example, if material scientists want to improve the rubber in an automobile tire, they may change chemical composition and organization at the molecular scale, but successful improvement of the material depends only on improvements in bulk constitutive relations. Such changes in properties could be achieved by a wide variety of molecular components and configurations; in other words, it may be possible to increase the rigidity of a polymeric material by increasing cross-linking between polymer chains or by increasing the density of polymers. However, because microscopic features are relevant only in their influence on large-scale parameters, the precise small-scale heterogeneity, molecular dynamics, and local nonlinearities are inconsequential.

Now let us imagine that these same material scientists want to construct a polymeric material capable of engulfing bacteria, much as a macrophage does. This is a challenging task. The energy scales characterizing the functions of a biological material are often comparable with thermal energies ($\sim k_B T$), and the length scales of structures are often comparable with those of component molecules (~ 5 nm for an actin monomer, ~ 50 -nm spacing between actin filaments in the leading edge of a crawling cell). At the cellular scale, the specific organization of the cytoskeleton can therefore play a key role in shaping relevant material responses. Energy consumption, in the form of filament polymerization or motor protein activity, can further enhance sensitivity to molecular fluctuations and actively drive structural reorganization. To emulate the macrophage, these material scientists must construct an active material, call it a polymerphage, that can sense the bacterium, follow it, and then engulf it on timescales faster than bacterial diffusion. In macrophages, this sequence of events happens much faster than the time required by the nucleus to orchestrate a response based on protein synthesis. Indeed, nucleus-free cell fragments formed from motile cells can themselves exhibit directed motility (**Figure 1**). As with the cytoskeleton (which drives both movement and capture in macrophages), the material composing the polymerphage must possess these adaptive capabilities as intrinsic material properties. The microscopic details of the polymer cannot be replaced with bulk material properties because the network is organized in a specific and hierarchical way to generate localized protrusions in the direction of movement toward the bacterium, to form new adhesions with the substrate to support the movement, and to disassemble the network away from the protrusion (10, 11). In addition, the local mechanical properties of the network vary spatially and change as protrusion, adhesion, and encapsulation modulate network density and microscopic constraints.

In light of the complex behavior and intricate organization of biological systems, describing a cell as a material of any sort may seem as inappropriate as describing a car as a material. But this analogy highlights the essential role context has in defining materials. It is indeed possible to compress a car and obtain a bulk modulus, just as it is for a cell. But to explain the reciprocating engine or the power windows in the car, such bulk properties are not instructive. Rather, we must identify the system of components that are involved in the process of interest and characterize their interactions. In this sense, one can understand the cytoskeleton as a system of interacting parts that collectively create material properties. The relevant features of these interacting parts are molecular in nature and are strongly modulated by mechanical cross talk. That these properties

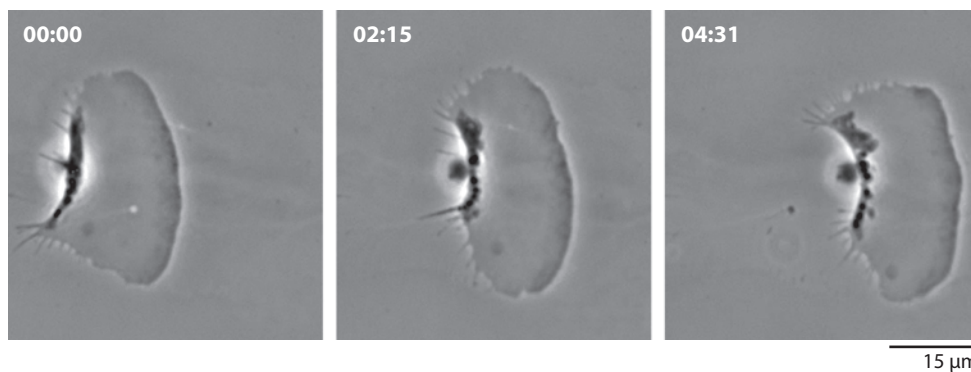


Figure 1

Crawling motility of a cell fragment containing no nucleus. Formed from a fish epidermal cell involved in wound healing, this fragment is able to crawl in a manner similar to the original cell. The active biological materials that drive this cell movement are capable of adaptive responses without nuclear control.

vary over time and space and in response to external signals presents experimental and theoretical challenges requiring diverse perspectives from chemistry, physics, engineering, and biology.

Given the complex interplay of spatial organization, the manner of perturbation, and nonequilibrium dynamics, it is critical to consider context when describing active biological materials. To illustrate this point with specific examples, we focus on the actin cytoskeleton and its relevant length scales and timescales, which requires the consideration of microscopic architecture, nonlinear response, and energy consumption. It is on these scales that the complex behaviors we most closely associate with living systems, such as motility, are operating.

3. IN VITRO RECONSTITUTION AS A TOOL FOR STUDYING ACTIVE BIOLOGICAL MATERIALS

The direct measurement of context-dependent material properties in cells is often complicated by the cell itself. Structures of interest and their dynamics cannot be separated easily from other mechanical events in the cell. The ability to directly isolate some cytoskeletal structures in active form has enabled the direct measurement of material properties, as in the case of stress fibers from adherent cells (12). In general, however, it is difficult to link molecular-scale mechanics and dynamics with behavior because molecular composition and organization are not fully known, even when a structure can be successfully isolated. A more direct route to understanding cytoskeletal structures in terms of their molecular components is to reconstitute them from purified proteins. Once genetic studies, electron microscopy, and live cell imaging have identified what are thought to be the key structural and regulatory molecules of a process, biochemical purification and reconstitution *in vitro* can be used to build a detailed physical description by directly rebuilding the structure.

The advantages of reconstitution are several. First, it can test whether the identified molecular components are necessary and sufficient to recapitulate a behavior. Remarkably, the relevant architecture of some cytoskeletal structures can be constructed from a small number of molecular species, as in the case of the reconstituted actin-based motility of the intracellular bacterial pathogen *Listeria monocytogenes* (13). Second, reconstitution enables direct biophysical interrogation of the structure to study material properties. Techniques including atomic force microscopy, micropipette aspiration, and optical tweezers can be used to measure physical responses. Third, reconstitution allows the role of regulatory proteins that control dynamics to be studied as perturbations on model materials. Although detailed studies of single molecules *in vitro* have successfully revealed the inner workings of individual mechanochemical enzymes such as myosin (14), it is not straightforward to predict collective properties of systems comprising many interacting molecules. By contrast, *in vitro* reconstitution provides the capability to study the emergence of micrometer-scale function from numerous molecular-scale interactions of active biological materials by investigating the impact of titrating different components. Once demonstrated *in vitro*, reconstitutions of biological structures face the question of whether they indeed represent the structures seen *in vivo*. Continued improvements in live cell imaging and biophysical measurement will help to answer this question and refine our definitions of the cellular functions under study. *In vitro* reconstitutions nonetheless present molecular systems whose organization and dynamics challenge our ability to provide physical explanations.

The phrase active material is sometimes used as a synonym for filament networks containing molecular motors such as myosin. By providing internal tension, these force-generating units can guide the specific reorganization of filament arrangements. They might additionally drive an equilibrium material into a steady state with significantly different mechanical properties (15). At a fundamental level, however, motors represent only one mechanism by which the biasing effect of energy input can render a material active. Here, consumption of ATP disturbs the detailed balance

of conformational transitions in such a way that motor movement proceeds sequentially with ATP binding, hydrolysis, and ADP release. In the absence of motors, ATP may still be hydrolyzed to impose a bias that drives networks far from equilibrium, but force generation need not be directly coupled to ATP hydrolysis as it is in molecular motors. During actin filament polymerization, for example, force is generated by the rectification of equilibrium fluctuations against a surface such as the cell membrane, creating a protrusion. Sometimes called a polymerization motor, polarized actin filament growth indirectly links ATP hydrolysis with steady-state assembly and disassembly of the polymer in a cycle that can do work, a process known as treadmilling (16).

The protrusive actin networks that drive membrane displacement during crawling motility can be explored in detail via reconstitution. Actin-based movement of micrometer-sized particles in both purified protein systems and cytoplasmic extracts has been used extensively to investigate both biochemical and biophysical questions about protrusive actin networks. These biomimetic motility assays are modeled after the actin-based movement of *L. monocytogenes*, which hijacks the host cell's cytoskeleton for movement (17). The surface protein ActA derived from *Listeria* has been shown to induce the polymerization of actin through the activation of the host cell's Arp2/3 protein complex—the same factor involved in actin network formation in the lamellipodia of motile cells (18). This protein, as well as surface-bound proteins that activate the Arp2/3 complex, can be purified and coated onto polystyrene beads or other inert particles, causing them to exhibit actin-based motility when immersed in cytoplasmic extract (19). Bead movement can be tracked over time with video microscopy, and bead velocity can be studied as a function of parameters such as bead diameter, bead shape, ActA surface density, and extract concentration (20–22). Reconstitution of actin network growth can also be achieved in configurations that allow the direct measurement of force and mechanical properties, such as from the cantilever of an atomic force microscope (Figure 2) (23, 24).

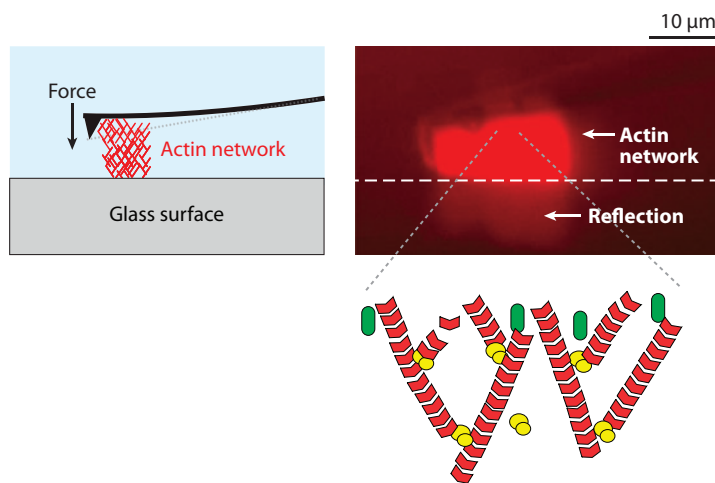


Figure 2

In vitro reconstitution of dendritic actin network growth. Cartoon and fluorescence image show labeled actin monomers in an actin network growing between a surface and the cantilever of an atomic force microscope. The growing network, similar to that at the leading edge of crawling cells, generates a force that displaces the cantilever, enabling the direct measurement of force and velocity. The dendritic actin network architecture is generated by molecules including actin monomers (red), nucleation promoting factors (green), and branching proteins (yellow).

Bead motility assays have served as the basis for the identification of a minimum motility system (i.e., a solution of purified soluble proteins that can replace cytoplasmic extract as the medium in which ActA-coated beads exhibit actin-based motility). Loisel et al. (25) reported a purified motility system containing four components—Arp 2/3 complex, actin, cofilin, and capping protein—in addition to ActA. Other actin-binding proteins (such as profilin, α -actinin, and VASP) influence actin network assembly and disassembly but are not necessary for movement in a low-force environment dominated by viscous drag. Although the number of necessary components is small, the material properties exhibited by reconstituted actin networks depend intimately on their organization.

In theory, there are many possible ways of spatially organizing the small set of proteins involved in dendritic actin network growth. The simplest is isotropic, e.g., the network of filaments generated by uniform mixing. Alternatively, some components could be spatially constrained, leading to the inhomogeneous assembly of filaments and anisotropic material properties. Variation in external mechanical or chemical inputs could further bias organization over time, leading to a material whose history determines its response. These more complicated constructions involving heterogeneous boundary conditions and time-varying biases most closely resemble the environmental constraints of the highly organized active biological materials responsible for cell movements.

4. ASSEMBLY: BUILDING ACTIVE BIOLOGICAL MATERIALS

Cells exploit molecular self-assembly, biased by energy consumption and boundary conditions, to create a diverse set of cytoskeletal structures. These structures, which must generate forces, resist deformation, and transmit stresses, span molecular to cellular length scales. For example, cortical actin networks provide a supportive shell beneath the cell membrane, whereas protrusive actin networks work to displace the membrane during motility (26, 27). Stress fibers containing large numbers of antiparallel actin filaments and contractile myosins bridge adhesion sites (28), whereas filopodia formed from a small number of parallel actin filaments create spike-like protrusions at the cell periphery (29). In each of these examples, elements of the cytoskeleton are assembled into a specific structure with mechanical properties and dynamical behavior unique to its function.

Protrusive actin networks in crawling cells provide an example of the complex interplay of length scales at work during the assembly of active biological materials. An actin filament is formed by the noncovalent assembly of actin monomers, each with diameter $d = 5.4$ nm, into a linear double-helix polymer with a typical length of ~ 100 nm. Individual filaments are then organized by a multitude of actin-binding proteins that cross-link, cap, sever, branch, and nucleate new filaments into dynamic and heterogeneous micrometer-scale networks (30). Protrusive actin networks, also called dendritic actin networks because of their branched structure, are characterized by highly anisotropic filament organization and the ability to generate directed forces for protrusion during motility, phagocytosis, and endocytosis (27).

Protrusive actin networks are formed between points of substratum adhesion and the leading-edge membrane of a crawling cell. For growth of the actin network to displace the membrane, the network must be stiff compared to the resistance to forward motion. Otherwise, growing filaments at the leading edge compress the internal actin network rather than extend the cell membrane in the crawling direction. The assembly of a mechanically stiff network that resists compressive forces is achieved by a sequence of steps governed locally by a small number of molecular interactions and globally by boundary conditions: When a protrusion is initiated, molecules on the cell membrane are first activated. The membrane-bound activated molecules then stimulate the attachment of the branching protein Arp2/3 to the side of an existing filament to nucleate growth of a daughter filament, with branch spacing along the mother filament of approximately 100 nm and a branch

angle relative to the mother filament of 70° (30–32). This daughter filament and the mother filament both elongate until their growth is permanently halted by a capping protein diffusing through the cytoplasm.

Critically, the confinement of the branching activation signal to the cell membrane creates a catalytically active two-dimensional surface that causes the dendritic actin network to be highly anisotropic. Only filaments that are elongating toward the membrane can continue to form branches because the activating protein can only trigger branch formation within a few nanometers of the surface. In contrast, the capping of filament elongation can occur anywhere in the network, acts to limit average filament length, and effectively localizes network growth near the nucleation surface. The net result is a highly branched network with a typical filament angle of $\sim 35^\circ$ to the surface. Repetition of these three simple steps—elongation, branching, and capping—creates a dendritic actin network that drives membrane displacement on the cellular scale.

The intrinsic organization of dendritic actin networks stands in contrast to cortical actin networks, in which filament orientation is less constrained, filament length is significantly longer, and filament density is reduced (26). Of these two, cortical networks more closely resemble bulk reconstitutions of randomly oriented and cross-linked actin networks (33). As we discuss in later sections, these different arrangements of filaments, cross-links, branches, and boundaries give rise to remarkably different material properties. This sensitivity to architecture highlights the central importance of context in describing active biological materials.

5. MECHANICS AND FLUCTUATIONS: PHYSICAL RESPONSES OF ACTIVE BIOLOGICAL NETWORKS

Systems that are heterogeneously and hierarchically organized can exhibit a dramatic variety of mechanical responses, depending not only on the strength, frequency, and wavelength of a deformation, but also on where it is applied. This situation is further complicated for active biological materials by nonstationary time dependence. The route to understanding any detailed mechanical interplay among these effects begins with considering them in the simplest contexts (e.g., measuring bulk elastic properties of an undeformed, isotropic network of purified filamentous actin at equilibrium). Rheological experiments on such simplified networks in the absence of cross-links have measured a linear elasticity of $E = 1$ Pa (34, 35), whereas experiments with cross-linked networks have measured a linear elasticity of $E = 1$ –100 Pa (33, 36–38). By contrast, living cells have elasticities in the range of $E = 100$ –5000 Pa (39–41). These discrepancies demonstrate that even simple mechanical properties of cytoskeletal materials can be sensitive to specific architectural details, including filament and cross-linker density, connectivity, and orientation.

The simplest mechanical properties of a material, and typically the easiest to measure, involve the linear response to applied forces such as the Young's modulus E . In equilibrium these susceptibilities reflect directly the nature and size of spontaneous fluctuations in corresponding mechanical variables (e.g., E and a system's length L). For systems driven out of equilibrium by motor activity or filament polymerization, this connection is less straightforward, but steady states should nonetheless satisfy a kind of regression hypothesis: Relaxation from conditions induced by an external force should be indistinguishable from appropriate spontaneous fluctuations about the undisturbed steady state (42). Linear response should therefore be determined by, and report on, typical excursions due to thermal excitations. For example, the Young's modulus measured for growing dendritic actin networks, $E = 1$ kPa, implies that typical fluctuations in total length L are of size

$$\langle \delta L^2 \rangle = \frac{k_B T L}{E A}, \quad (1)$$

where A is the cross-sectional area. Highly nonlinear response, by contrast, may be determined by rearrangements that are unlikely prior to the perturbation. Which regime is relevant depends on the magnitude of the applied force relative to thermal energies and characteristic length scales.

Forces experienced by actin networks in biological contexts vary significantly in strength. A single filament can generate ~ 1 pN as it polymerizes against a load (43, 44), whereas a single molecule of myosin can contract with a force of ~ 5 pN (45). Collectively, the actin network formed at the leading edge of crawling cells has been shown to produce forces above 1 nN along a 1- μm region of the edge (46). During phagocytosis, macrophages have been shown to generate membrane tensions as high as 1 mN m^{-1} (47). The local and global deformations of the cell resulting from these internal force generators and external boundary conditions depend on the material response of the cytoskeleton.

The statistical mechanics of cytoskeletal response can be quite rich even for the simplest reconstitutions at equilibrium. On the microscopic scale, individual filaments respond to extension and compression in different ways, both highly nonlinear. Similar to a simple flexible chain molecule, F-actin contracts from an extended state at equilibrium due to entropy. The number of thermally accessible microstates decreases sharply as a chain's end-to-end distance approaches its contour length, so that the entropic elasticity of a marginally flexible chain (such as a 100-nm actin filament in which the persistence length is $\sim 12 \mu\text{m}$) is strongly anharmonic. Under compression, such a filament behaves much like an elastic rod at zero temperature and is extremely susceptible to collapse at forces greater than a threshold for buckling. Indeed, there is evidence that both filaments in extension and filaments in compression are important in the mechanical response of reconstituted actin networks. Cross-linked actin networks exposed to shear stresses exhibit nonlinear stress stiffening, consistent with a response dominated by filaments in extension (33, 34, 36–38, 48). By contrast, dendritic actin networks exposed to compressive stresses exhibit stress stiffening followed by reversible stress softening at high loads, consistent with a response dominated by filaments in compression at high stresses (24).

Networks in which semiflexible filaments are coupled via cross-links, branches, or strong entanglements naturally bring the nonlinear responses of extended and compressed filaments into competition because the extension of one chain could easily necessitate the compression of its neighbors. This notion suggests an important role for collective fluctuations, mediated by constraints involving multiple filaments. Several studies have proceeded with the alternative idea that the mechanical response of an entire network can be inferred directly from that of an isolated filament. Although certain scaling properties of experimental measurements can be reproduced in this fashion, the generality of such simplifications awaits confirmation from calculations that carefully represent both intrachain nonlinearities and interchain constraints. Simulations from such a model (**Figure 3**) indicate heterogeneous strains in a filament network at different time points.

The potential importance of highly collective fluctuations can be illustrated by considering the growth of an actin network abutting a rigid obstacle. Oster and coworkers (49–51) have explained how energy input can rectify symmetric fluctuations in such a system and thereby generate directed force. One version of their Brownian ratchet model imagined a single actin filament tethered to a stationary network, situated sufficiently close to the obstacle so that adding new monomers of size d to the filament's end requires a rare fluctuation. The typical rate of isolated filament bending motions with amplitude $d/2 = 2.7 \text{ nm}$ (which is the increase in contour length due to the addition of one monomer into the double-helix chain) then serves as an estimate for the network's local growth kinetics. On the micrometer scale, coordinated fluctuations of the network have the potential to influence growth. Indeed, for a dendritic network of average size $L = 1 \mu\text{m}$ in the direction of growth, $E = 1 \text{ kPa}$, and cross-sectional area $A = 1 \mu\text{m}^2$, Equation 1 yields $\sqrt{\langle \delta L^2 \rangle} = 2.2 \text{ nm}$, so that fluctuations of magnitude $d/2$ are quite commonplace. Although

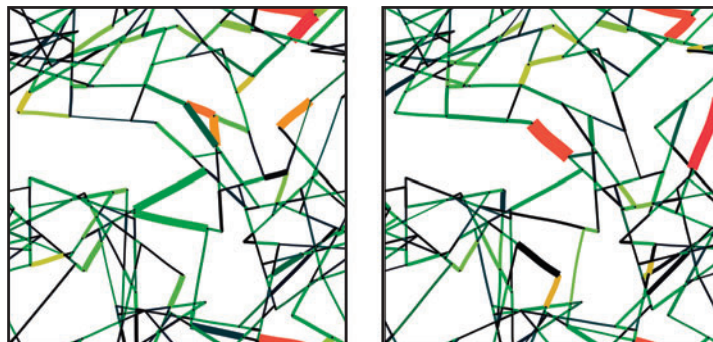


Figure 3

Two snapshots from a computer simulation of an elastic network comprising cross-linked semiflexible polymers. Line colors and thicknesses indicate each segment's instantaneous longitudinal and transverse strain, respectively (with *thick red lines* representing the strongest deformations). Although these two configurations share some similarities in the way stress is distributed through the system, they also possess marked differences. These images demonstrate that in the course of natural thermal fluctuations, such a network accesses many distinct microscopic strain states on the length scale shown. Moving among these states requires collective redistributions of force. Fluctuations of this nature, neglected in both continuum and single-chain theories of actin networks, could contribute importantly to dynamical processes, such as elongation at the leading edge of a growing network or the initiation of a filopodium, that are sensitive to mechanical variations over a wide range of length scales.

cross-linked regions of the network may be locally quite stiff, the flexibility that accumulates over large regions can be considerable.

This Brownian ratchet model illustrates an important aspect of actin networks as active materials. Behaviors on widely different scales that are tempting to consider independently can become closely linked by nonequilibrium dynamics. Existing theoretical descriptions of growing networks tend toward either a discrete extreme, focusing on individual filament dynamics and single monomer addition, or a continuous extreme, casting a large collection of filaments as a featureless viscoelastic medium evolving smoothly in time. That these disparate approaches each capture certain nontrivial aspects of observed phenomenology suggests a truly multiscale character for network dynamics. It is likely that a comprehensive description will require attention to the mutual interdependence of the microscopic reorganization of molecular structure and long-wavelength and long-timescale redistribution of stress.

The difficulty of compactly describing the mechanical response of an active biological material is compounded further still by ambiguity in partitioning external and internal forces. Motor proteins, for example, can greatly enhance the stiffness of isotropic cross-linked actin networks, perhaps endowing them with elasticity comparable with that of living cells (15, 33). Considering these molecules as integral components of the system, one might associate the nonlinear differential elastic stiffness induced by prestress with the linear response of a biologically relevant material. The internal stress necessary to achieve kilopascal stiffness in a prestressed network, however, poises the reconstituted network very close to rupture. As a result, the regime of linear response here would be extremely limited. It is similarly unclear whether a lipid membrane in contact with a growing dendritic network should be thought of as a part of the material, as a boundary condition, or as a complex perturbation. As we discuss in Section 7, the dynamic coupling between network and membrane can drive rearrangements that are highly atypical of either system in isolation.

Blurred distinctions between the system and its surroundings appear inherent to the way living systems put active biological materials to use. The mechanical responses most useful to a cell are

often weakly related to those induced by smooth deformations of separated cytoskeletal components. They instead involve heterogeneous perturbations, both biochemical and mechanical in origin, that access a wide variety of nonlinear behaviors. It is therefore difficult to imagine describing the biologically relevant material properties of actin networks in any simple unified way, for example, in terms of a single stress-strain curve. The vast space of parameters defining such complex perturbations argues strongly for studying processes such as lamellipodial protrusion, filopodial protrusion, phagocytosis, endosomal rocketing, stress fiber contraction, endocytosis, exocytosis, and membrane receptor reorganization under conditions that closely mimic the physical context of function *in vivo*.

6. DYNAMICS: TIME-DEPENDENT BEHAVIOR OF ACTIVE BIOLOGICAL MATERIALS

The chemical and mechanical forces that drive active materials out of equilibrium *in vivo* can vary significantly in time. Such changes may be regulatory in nature, controlled from within the cell to program particular behaviors. Alternatively, they may constitute extracellular signals, often requiring responses more rapid than can be orchestrated by the nucleus. In either case the dynamics of large-scale reorganization becomes an important material property for cellular function. The biologically relevant collection of such properties need not be encoded simply in fluctuations about equilibrium or steady states, possibly depending instead on a diverse set of highly nonlinear time-dependent responses.

Cytoskeletal networks operate over a wide range of timescales. For an actin monomer concentration of 10 μM , free actin filaments elongate at a rate of ~ 100 subunits per second, equivalent to a velocity of $\sim 0.3 \mu\text{m s}^{-1}$ (27). The presence of load reduces this elongation rate exponentially (52), but growth can also be accelerated by the presence of certain actin binding proteins such as formins (53). Myosin II molecules from smooth muscles move at rates of 250 nm s^{-1} , whereas isoforms from fast skeletal muscle can drive contraction at rates as high as 8000 nm s^{-1} (54). Adhesion between cells has been observed to strengthen over the course of an hour owing to cytoskeletal remodeling (55). This mixture of timescales both enriches material responses and greatly complicates their prediction.

Only in special cases can the response to time-varying perturbations be straightforwardly connected to features of stationary states. Most simply, changes in control parameters or driving forces could be applied over timescales much longer than intrinsic relaxation times. Here one can reasonably expect adiabatic evolution from one stationary state to another, in which the system never leaves a manifold of well-defined equilibrium or steady states. Understanding this gradual response requires only knowledge of a few macroscopic susceptibilities and constitutive relations. Dynamics is also greatly simplified in the regime of linear response. In this limit, the evolution induced by any combination of time-varying perturbations can be constructed simply as a superposition of impulse responses. The frequency dependence of corresponding Green's functions then provides a complete picture of the timescales for relaxation under external forcing. In simple reconstituted actin networks, this has been shown to be the case (56), but few measurements have tested this in nonequilibrium steady states.

Theoretical treatments of actin networks, and even whole cells, as continuous viscoelastic media combine aspects of these approximations. It is imagined that, as in conventional materials, every mesoscopic region of the network is fully characterized by its local stress and velocity. The inclusion of a single phenomenological relaxation time effectively asserts that all remaining degrees of freedom respond linearly, and instantaneously. The mean field evolution of stress and velocity fields can then be propagated by partial differential equations analogous to those of macroscopic

hydrodynamics. When active stresses due to polymerization and motor forces are incorporated, their solutions yield rich phase diagrams for nonequilibrium steady states and detailed predictions for network growth kinetics (57). None of the approximations we have described as underlying these continuum approaches, however, is guaranteed for cytoskeletal materials by known separations of timescales (e.g., between local stress relaxation rates and the frequencies of other modes for microscopic rearrangement) or length scales (e.g., between the biologically relevant system sizes and the distance over which correlated stress fluctuations decay at equilibrium). Establishing the limits of their accuracy is an important goal for molecular simulations that can bridge such length scales and timescales.

For the actin cytoskeleton, we have observed instances in which continuum approximations break down in even simple reconstituted systems. When load on a growing network, applied by the cantilever of an atomic force microscope, is increased slowly, filament density increases smoothly and growth velocity remains constant (23). This response appears to be adiabatic in nature, leading one to expect that the network's steady state is uniquely specified by a handful of external mechanical and chemical parameters (e.g., load, concentration of monomeric actin, and cross-sectional area). Similar behavior has been observed in live cell studies (46). Perturbations that are more sudden than intrinsic relaxation, however, reveal a much richer set of possibilities. When force is slowly increased by growth of the network but returned to its initial value within 2 s by stepping the cantilever of the atomic force microscope away to reduce its deflection, the network relaxes to a steady state different from the one in which it began. In particular, it features a significantly elevated growth velocity and presumably higher density at the leading edge. This history dependence serves as a strong reminder that the way biological materials are handled can strongly alter their properties.

For the purpose of inferring properties of active materials from the behavior of related equilibrium systems, a lack of uniqueness in steady states is problematic. In the example of dendritic actin growth under time-varying load, it is not clear whether the two steady states could be related to two equilibrium networks that differ through a static control parameter, or whether instead their mechanical properties differ in fundamental ways from those at equilibrium. In the world of conventional materials, the multiplicity of apparently stationary states is a hallmark of glassiness. Although the microscopic structure of such systems is often indistinguishable from that of parent disordered liquids, their mechanical responses are dramatically different. One would be hard-pressed to predict from the dynamics of the nearby disordered state that such pathologically slow relaxation should arise.

Analogies between glasses and active biological materials have been proposed and explored at a phenomenological level. Cells can indeed exhibit characteristically glassy behaviors such as fluidization and time-temperature superposition (58, 59). It is not clear how precisely ideas such as kinetic facilitation and caging that have clarified the basic physics of conventional glasses apply to the cytoskeleton at a microscopic level. Such comparisons do, however, usefully emphasize the importance of context and correctly anticipate a diverse range of timescales relevant for material behavior. Just as the liquid state can serve as a misleading reference system for a glass, reconstituted networks that lack sensitivity to history may yield limited insight into certain biological functions.

7. INTERPLAY AMONG ASSEMBLY, MECHANICS, AND DYNAMICS IN ACTIVE BIOLOGICAL MATERIALS

Above we discuss the major properties of active biological materials separately. However, in any biological context, these properties become interdependent on multiple levels. Here we describe two experiments in which the interplay between assembly dynamics and mechanical response

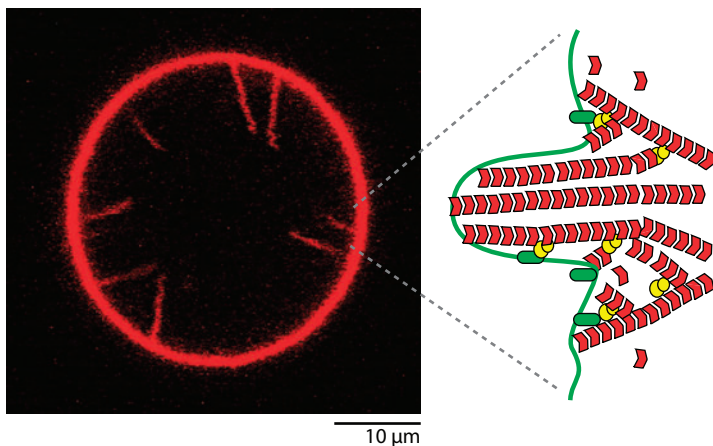


Figure 4

Parallel actin filament protrusions against a bilayer membrane. A confocal fluorescence image shows narrow actin protrusions emerging from a reconstituted dendritic actin network growing against the outer surface of a giant unilamellar vesicle. Interactions between the dendritic network and the deformable bilayer membrane lead to the clustering and parallel growth of the membrane-bound filopodium-like protrusions.

yields unexpected behavior. In both experiments, a reconstituted dendritic actin network was grown against a lipid bilayer membrane, emulating interactions at the leading edge of a crawling cell. Although it is well-known from studies of dendritic actin networks against hard surfaces that polymerization alone can generate force, it is unclear how the deformability and fluidity of the membrane influence the organization of the reconstituted actin network and how, in turn, the actin network influences the organization of the membrane (60).

In the first example, a minimal set of proteins required to generate a branched actin network was grown on the outer surface of a giant unilamellar vesicle (61). As a dense network assembled the membrane, occasional spike-like protrusions emerged from the interface into the vesicle (**Figure 4**). Each narrow ($d < 0.5 \mu\text{m}$) protrusion contained long actin filaments that were fully enclosed in a membrane tube, polymerizing at the tip, with no apparent branching along the length. Such a structure shares a striking and unexpected resemblance to filopodia, the spike-like parallel actin filament structures that are generated at the edge of motile cells such as neuronal growth cones (62). According to the convergent-elongation model of filopodial formation in cells (63), dendritic actin networks give rise to filopodial protrusions with the assistance of two additional factors: a tip complex that binds the growing ends of adjacent filaments and a bundling protein, fascin, that laterally stabilizes actin filaments. However, the experiments contained neither of these proteins.

How could filopodia-like protrusions emerge from a dendritic network without any additional proteins? We suggest that interactions between the elastic membrane and the growing actin network carry out the functions of gathering filament tips together and stabilizing parallel filament protrusions. First, the energetic cost of small membrane deformations created by the growth of individual filaments is minimized by filaments bending to congregate within a single membrane deformation. Second, the gathered filaments continue to elongate (in the absence of capping protein) and deform the membrane until it forms a narrow tube around the aligned filaments. Finally, the membrane tube stabilizes the actin filaments against collapse, permitting further elongation until the reaction goes to completion or the protrusion encounters resistance, at which point it can buckle (64). This example of filopodia-like structures emerging from a dendritic actin network shows the importance of context and the role that rare fluctuations can have in defining cell shape.

In the second example, a similar experimental system was used to study the spatial organization of lipids within the membrane of an actin-associated giant unilamellar vesicle (65). Different from the homogenous membrane in the previous example, a tertiary mixture of lipids capable of phase separation in the absence of actin was used. At temperatures above the miscibility transition temperature (T_m), a reconstituted actin network grew uniformly around the vesicles as above. When the membrane was cooled below T_m , the lipids partitioned into liquid ordered and liquid disordered domains. If purified proteins were added to a phase-separated vesicle, the reconstituted dendritic actin networks would nucleate only on the liquid disordered domains containing the lipid activator of dendritic actin network growth. When the actin-associated and phase-separated vesicles were then heated, the temperature required to drive the mixing of the phases was several degrees above T_m . This shift in temperature suggests that the actin network, through its tethering to a low-concentration lipid species, stabilizes the phase-separated domain against the entropy of mixing. To directly test the implications of this temperature shift, we initiated actin polymerization on vesicles held close to but above T_m . The polymerization of the actin network on the membrane was sufficient to drive phase separation of the membrane, with no change in temperature of the system. This result demonstrates how actin network assembly and membrane fluidity collaborate to determine the organization of both network and membrane components.

8. CONCLUSIONS AND OPEN QUESTIONS

Descriptions of cells as materials can provide mechanistic insight into their movements and shape changes. In the simplest case, linear response functions require no specification of the strength or time dependence of applied forces. Scaling relationships (e.g., between applied stress and nonlinear compliance) similarly offer a broad view of physical behavior, perhaps spanning many decades of length, force, and time. Such properties report on a system's basic organization and the nature of its typical fluctuations when undisturbed. They can supply a road map for anticipating how a material will be sensitive to the wavelengths and frequencies of simple perturbations. However, as argued above, measurements and theories of these quantities, while providing a useful starting point for understanding active biological materials, by themselves paint an incomplete picture.

Some of the most important features of biological structures, such as protrusive actin networks and contractile stress fibers, involve transitions between distinct structural states. It is in this capacity that cellular subsystems can act as sensors, arbiters of large-scale adaptation, and agents of change in the life cycle of a cell. Fluctuations that mediate dramatic change in the physical world are rarely well presaged by the placid dynamics of initial or final states. A simple example from the physical world is evaporation. Even a thorough characterization of density fluctuations of the metastable liquid would, by itself, provide little insight into the mechanisms of nucleation and growth that ultimately guide the system through a transition state to the new macroscopic phase. Understanding this property of a fluid requires attention to the statistical mechanics of an extremely rare event. In principle, these rare events can be made more common by applying appropriate driving forces. For the case of a liquid, this amounts to superheating above the boiling point. For a cell, this might involve specific binding proteins that act, for example, to bias filament organization into a contractile stress fiber.

Understanding the microscopic mechanisms by which growing actin networks switch between nonequilibrium states calls for a new generation of theoretical models. When the nature of collective coordinates underlying the dynamics of interest is not known a priori, one has little choice but to represent explicitly a large number of microscopic degrees of freedom. By contrast, most existing approaches to cytoskeletal mechanics do not attempt to bridge molecular and system length scales

but instead assert the exclusive relevance of individual filament fluctuations or continuous elastic modes. We thus expect it will be necessary to combine features and length scales of several previous approaches. In the case of filopodial growth, the narrow dimensions of the membrane tube require explicit consideration of individual filaments dynamics, as in Brownian ratchet models. Restoring forces of the membrane implicate contributions from multiple filaments in the rare fluctuation initiating protrusion. Coupling among these filaments is mediated by the network as a whole. As a first approximation one could consider the network to be a viscoelastic continuum, although substantial heterogeneity in filament density at this length scale indicates that a more detailed representation of network mechanics is needed, as in calculations that solve for elastic ground states of cross-linked semiflexible rods. Fluctuations neglected in these treatments, however, may be important in shaping membrane rearrangements. It appears that an explicit representation of membrane mechanics would be crucial in this example.

Ultimately, appropriate models must embrace the thoroughly nonequilibrium character of active biological materials. Schematic theories that focus only on this feature provide some general insight into the stabilities and rigidities of steady states (66). Lacking the polymeric basis of cytoskeletal elasticity, however, they are not designed for exploring the kinds of specific phenomena discussed in this review. For this purpose, it is likely wiser to model explicitly the forces due to motors and polymerization that drive biological materials away from equilibrium. We therefore have in mind models whose threshold of microscopic resolution is set by length scales and timescales of basic events, such as monomer addition to growing filaments, and whose overall extent is comparable to the length scales and timescales of model experimental systems (67, 68).

In describing active biological materials, we use the actin cytoskeleton and some of the structures it forms as a rich source of examples. However, cells make use of a wide variety of molecular systems to animate their movements, and numerous other examples exist that stretch our definition of a material. These include the networks of microtubules involved in chromosome separation, intermediate filaments that contribute to the elasticity of cells, and the extracellular matrix that connects cells within tissue (69–71). Further examples arise in bacteria where cytoskeletal filaments play roles in cytokinesis as well as chromosome and plasmid segregation (72). Although the era of vitalism is long past, a tendency still exists to attribute the complex movements of cells to equally complex control systems somehow distinct from the machinery driving those movements. Research now shows that these materials possess an inherent capacity to evolve over time and exhibit new properties in response to stimulus, without control from the nucleus. Although genetically controlled changes in protein concentration and regulatory signals can and do influence the physical behavior of cells, active biological materials need no brain to guide their adaptive responses.

This review focuses on the unique characteristics of active biological materials that make them a fascinating topic of study. Three in particular stand out. First, active biological materials are not spatially uniform. They are highly organized structures whose bulk composite material properties, similar to that of a car, are not informative of their inner workings. Second, unlike a car, active biological materials are not macroscopic. They are molecular in scale, similar in size to the molecules that serve as their engines and wheels. Fluctuations can therefore be important for describing their emergent dynamics. Third, active biological materials are not simple mechanical systems. The function of a structure within a cell can depend on dramatic changes in state, whereby nonlinear dynamics along specific reaction coordinates determine behavior. Only by knowing precisely how such a mechanical process works can we decide the relevant physical interactions. Exciting progress has been made in recent years. With further studies, it may be possible to uncover the remarkable ways in which biology has succeeded in integrating material responses with molecular controls to create behavior.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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