Crawling Toward a Unified Model of Cell Motility: Spatial and Temporal Regulation of Actin Dynamics

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Abstract Crawling cells of various morphologies displace themselves in their biological environments by a similar overall mechanism of protrusion through actin assembly at the front coordinated with retraction at the rear. Different cell types organize very distinct protruding structures, yet they do so through conserved biochemical mechanisms to regulate actin polymerization dynamics and vary the mechanical properties of these structures. The moving cell must spatially and temporally regulate the biochemical interactions of its protein components to exert control over higher-order dynamic structures created by these proteins and global cellular responses four or more orders of magnitude larger in scale and longer in time than the individual protein-protein interactions that comprise them. To fulfill its biological role, a cell globally responds with high sensitivity to a local perturbation or signal and coordinates its many intracellular actin-based functional structures with the physical environment it experiences to produce directed movement. This review attempts to codify some unifying principles for cell motility that span organizational scales from single protein polymer filaments to whole crawling cells.

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INTRODUCTION

A crawling cell achieves its movement by protruding its front and retracting its rear to displace itself directionally. This type of amoeboid motility is the mechanism by which many different cell types, including slime molds, amoebas, leukocytes, fibroblasts, epithelial cells, and neuronal growth cones, fulfill their specific biological roles. Although these cells’ overall migration occurs through related molecular and cellular mechanisms, their types of movement are very distinct and are optimized to their specific environments. Consider three examples of crawling cells moving in different environments with very different morphologies: the keratocyte, the neutrophil, and the neuronal growth cone [see the supplemental online movies 1, 2, and 3 (follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org) and the section Coordination of Different Properties of a Cell to Produce Movement below]. Epithelial keratocytes function in wound healing, in which cells at the outer edge of an epithelial sheet move along the surface toward the wound as the cells deeper in the sheet follow (1). The keratocyte glides elegantly along its two-dimensional environment by protruding a single thin lamellipodium and retracting its rear in a highly coordinated manner. In contrast, a neutrophil intensely pushes its way through three-dimensional tissues to locate and consume invading microbes (2). It follows its target by extending thick pseudopods in many directions as it responds and adapts to a rapidly changing environment of external signals. Finally, the neuron extends a specialized structure, the growth cone, to find distant targets in the wiring of the nervous system. The growth cone explores its environment by extending dynamic filopodia and migrates along preestablished molecular paths laid out in the developing embryo (3). The protrusion-retraction mechanism in each of these cell types occurs through the assembly of complex dynamic organizations at the front and their coordinated disassembly at the rear of the cells. They each achieve their protrusive dynamics through the polymerization of actin and the regulation of higher-order multicomponent actin structures. Their molecular components have similar functionalities,
which include proteins stimulating actin nucleation, enhancing actin polymerization, accelerating actin depolymerization, and regulating the structural organization and dynamics of the complex actin networks in the protruding cellular regions. Yet the coordinated effort of all of these similar molecules, in the context of their specific cell type, produces very distinct morphologies during each cell’s migration.

The tremendous advances in molecular biology and biochemistry that have occurred in the past 20 years have allowed researchers to begin identifying members of the long inventory list of individual proteins and structures contributing to crawling cellular motility. Their efforts have lead to the classification of specific protein interactions and their regulation within subcellular modules of cellular motility, such as the protrusion of lamellipodia and filopodia, adhesion to the substrate, and retraction of the rear. Now that classification and characterization of small subcellular modules of cell motility are well underway, a new challenge has come to the fore—understanding how the cell coordinates these modules to achieve robust whole-cell motility that is responsive to mechanical and chemical features of its environment. The cell must regulate its overall diverse biological functions within each hierarchical level beginning with the behavior of individual proteins at the nanometer size scale yet translating to behaviors, four orders of magnitude larger, over tens of microns at the level of the cell. Protein-protein interactions are dynamic over timescales of milliseconds to seconds, yet whole-cell motility can remain persistent for minutes to hours, four orders of magnitude longer. The cell must exert exact control over the nonadditive layers of complexity that such vast differences in size and timescales create. This review explores how the cell can regulate each of these levels, from individual molecules through the combinatorial nature of their interactions that create dynamic structures to the global behavioral changes mediated by these structures and needed for the cell’s specific biological role. Several of the basic unifying principles necessary for cell motility are examined. One such principle is a “treadmilling” at all levels of the cell with assembly at the front and disassembly at the rear through which directed movement occurs. Additionally, the cell regulates transitions between dynamic subcellular self-organizing structures to be sensitive yet robust in its response to the environment. Finally, the cell coordinates these distinct structures and components in space and in time within its crowded, yet highly structured, cytoplasm, such that it can leverage their integrated functions to produce its movement.

EXPERIENCING THE CELLULAR ENVIRONMENT

One major challenge in taking information learned in simplified biochemical systems and applying it to the living cell is that the physical properties within the cell itself differ significantly from conditions in dilute aqueous solution. The cell’s viscoelastic environment renders inertia insignificant (4), and the mech
ical properties of cytoplasm are dependent on the speeds of physical deformations within the cell. The large number of subcellular structures, organelles and macromolecules creates a crowded environment where ability to move is strongly dependent on the size of the individual component. Movement over distances that are large relative to the size of the component cannot occur simply through diffusion (5). The cell therefore requires organized transport mechanisms to regulate the spatial distribution of its components in time. The spatial boundary created by the cell plasma membrane and typical cell volume of picoliters reduce the meaning of the molar concentration of a specific molecule; so it is important to consider the finiteness of its numbers, unlike in vitro solutions with a volume of microliters or milliliters. Fluctuations caused by thermal energy or inherent noise can be significant in effecting the outcome of a cellular process, for example by changing the immediate position of a molecule and, because of its finite numbers within the cell, considerably changing its distribution in the local environment. Appreciating the complexity and nonclassicality of the environment that any intracellular component experiences is fundamental to integrating information about these components and to understanding the global behavior of a cell as a whole.

Cells contain significant mass percentages of protein, which sometimes exceed one quarter of their weight. The large proportion of proteins in the cytoplasm implies that their intracellular distribution more closely resembles a protein crystal than a protein solution (6). Taking the sizes of the proteins and the other macromolecules in the cell into account, as well as the structures into which they can assemble, leads to a rather crowded and inhomogeneous view of the intracellular milieu. Goodsell’s (7) correctly scaled depiction of the Saccharomyces cerevisae cytoplasm and its components, including the sizes and concentrations of soluble proteins, ribosomes, and cytoskeletal structures (Figure 1a), demonstrates the crowding well. Recently, advances in microscopy have allowed the three-dimensional visualization of Dictyostelium discoideum cytoplasm via cryoelectron tomography (Figure 1b) (8). The similarity in the spatial distribution of cytoplasmic components between these two images is striking and, although each includes several different components and spans different size scales, clearly demonstrates the degree of cytoplasmic crowding at all levels within the cell. Diffusion rates for small tracer molecules in fibroblast cytoplasm and 12% to 13% dextran or Ficoll solutions are similar; this observation leads to an estimate that only half of total cytoplasmic protein is in solution at any given moment (9). Interestingly, observations of the statistically lower than expected variability in isoelectric points and molecular weight distributions of the proteome dating back almost 20 years also led to suggestions that up to half of proteins may be confined to structures and not free in solution (6), a fact currently well accepted in light of more recent ultrastructural investigations of the cytoskeleton. An attempt to create interaction maps of the yeast proteome combined and analyzed extensive two-hybrid data and found only one large network of protein interactions linking over 1500 proteins in the proteome,
whereas all of the other networks contained fewer than 20 proteins (10). Individual proteins are thus highly interconnected in their interactions and are spatially regulated by their mutual interactions with the structural scaffold of the cell, demonstrating the highly ordered nature of the intracellular environment.

The crowded nature of the cytoplasm affects the properties of molecular interactions by favoring the folding of proteins, intramolecular associations, and the formation of oligomeric structures [reviewed in (11, 12)]. The size of a macromolecule and the presence of other macromolecules nearby reduce the volume and components from the local environment accessible to that macromolecule. The effective concentration of neighboring molecules is therefore increased and depends on their relative sizes. The rate-limiting step in a reaction can depend on the ability of two molecules to find each other in space or on the kinetics of their specific association, which determines the respective positive or negative effect of crowding on a cellular process (11, 12). Experiments have shown that crowding is the principal factor in decreasing diffusion of a small probe, while binding interactions and the microviscosity calculated in the absence of other interactions (fluid-phase viscosity) are not. In fact, the fluid-
phase viscosity of small solutes is only 10% to 30% less in cytoplasm than in water [reviewed in (13)]. Although diffusion can be the principle mechanism of molecular movement for interactions on a \( \sim 20 \) nm scale, it cannot completely explain the mechanism by which molecules move over greater distances to perform their functions (5). It is therefore crucially important for the cell to regulate the spatial distribution of its proteins very tightly to control its functions and to foster mechanisms other than diffusion for long-distance transport of large components including protein oligomers.

One consequence of intracellular crowding is a size-dependent diffusion capability of tracers in cytoplasm indicative of a molecular sieving mechanism, probably owing to the actin cytoskeleton [reviewed in (9)]. The dense cytoplasmic meshwork of actin filaments excludes larger tracers as well as intracellular organelles from regions of the cell, including lamellipodia and pseudopodia (14), and accounts for most of the diffusional constraint within *Dictyostelium* (15). Specialized mechanisms to transport molecules to the cellular regions where they function are therefore necessary, especially when those regions are within a dense actin meshwork, such as at the leading edge of crawling cells. It has been shown in two enzymatic systems that crowding itself may increase sensitivity to the environment [reviewed in (16)]. In a region of high macromolecular or structural density, crowding decreases the absolute numbers of a specific molecule that are able to access that region and could make the region more sensitive to fluctuations in the spatial distribution of the molecule. Inherent noise and fluctuations within the cell provide opportunities for stochastic amplification of a process beyond an easily reversible state, leading to many possible outcomes in a signal-sensitive cell. Thus the physical properties of the cellular environment significantly contribute to the cell’s overall spatial and temporal regulation of its components, especially in the dynamics of its coordinated movement.

**TREADMILLING AT ALL LEVELS OF CELL MOTILITY**

The basic unit of motility must be able to protrude in the direction of motion and retract in the opposite direction to displace itself from one point to the next. This basic principle applies to all levels of motility within the cell, from the treadmilling action of a single actin filament to the treadmilling of a large motile cell coordinating all of its components to protrude in the front and retract in the rear. To understand the mechanism by which a cell can displace itself in space, we must consider the mechanisms of displacement at all spatial and temporal scales therein.

**The Inherent Asymmetry of Polymers**

In considering basic principles of biological assembly, Crane (17) noticed that building a chain from a set of asymmetric objects in which each object can only interact with another in a specific spatial orientation created structures of a helical
nature, regardless of how different the objects were in shape and size. Pauling (18) took this concept and applied it specifically to protein polymers. A single globular protein is always asymmetric. Therefore, if a protein is capable of interacting with an identical or distinct binding partner, the resulting dimer and subsequent multimers will preserve this asymmetry and form a helical polar polymer. The variety of possible polymer structures depend on the diameter of the protein, the pitch of the helix induced by polymerization, bond flexibility and geometry, and the energetic preferences of the protein subunits for interactions along different surfaces. Multiple individual polymer helices can interact with each other to stabilize their resulting structure (Figure 2a) (18). This basic concept explains the helical nature of various polymeric cellular structures as well as how the specific shapes of their subunits lead to different geometries, which includes the double protofilament helix of actin (Figure 2a), and the higher number of protofilaments in microtubules and flagella (19).

**Combining Actin Filament Structural Asymmetry with Distance and Time**

The globular asymmetric nature of the monomeric actin subunit itself thus creates the basis for the helical nature and structural polarity of a single polymeric actin filament. In this case, energetics would dictate that the ratio of the on and off rates between monomer and either end of the polymer must be equal. However, the structural asymmetry of the filament causes different specific interactions at each end that result in one (the barbed front end) having much faster kinetics than the other (the pointed back end). In order to transform the structural polarity of actin into a dynamic spatial structure, a temporal regulator is needed to translate the asymmetric kinetics inherent in the filament structure into asymmetric energetics (20). For actin, association with ATP and its subsequent hydrolysis provide this clock. The energy released by the nucleotide hydrolysis can induce a conformational change in the protein, which alters the associations between a subunit and the rest of the polymer based on the state of the bound nucleotide. A polymerization system that is differentially sensitive to the relative numbers of ATP- or ADP-bound subunits at each end is thus created. This coupling to a tiny clock regulates the temporal dynamic behavior with different energetics at each end [reviewed in (21)]. As a consequence, within a range of free monomer concentrations, the front end can polymerize while the back end depolymerizes causing a displacement in space, a process called treadmilling (Figure 2b) (22). This process has been observed for individual actin filaments in vitro (23) as well as for microtubules in vivo (24, 25).

**Accelerating Treadmilling in Time and Expanding It in Space: The Actin Array**

Actin filament dynamics are regulated spatially, through the intrinsic polarity of an asymmetric protein polymer, and temporally, through coupling this asymme-
try to nucleotide hydrolysis, which together create the basic unit of motility, a treadmilling filament. At monomer concentrations similar to those inside cells, treadmilling is extremely slow (23, 26). The behavior of a single filament is therefore insufficient to span the large size of the cell or produce the rapid polymerization/depolymerization dynamics required for cellular movement. The

Figure 2  Treadmilling as considered at all levels of the cellular organizational hierarchy. (a) A protein polymer made of asymmetric globular subunits has an intrinsic helical nature. Two of these helical polymers can interact to form higher-level structures stabilized by their associations. The top shows an asymmetric globular protein subunit (I) that upon iterative self-association forms a helical polar polymer (II). Two of these helices may interact and form different structures depending on their specific intermolecular interactions, as seen in (III) and in the reconstruction of an actin filament on the bottom. (b) Leveraging the polar nature of the polymeric protein helices by adding a time-dependent variable to its association, the hydrolysis of nucleotide, provides the basis for the treadmilling of a single actin filament. This basic unit of motility can assemble at the front and disassemble at the rear, between two barriers (top) or along a surface (bottom), effectively moving an individual filament forward, and can produce force through its polymerization. (c) The regulation of actin treadmilling behavior in assembling dynamic protruding regions of the cell occurs by complex combinatorial control of its dynamics through the action of accessory proteins. This figure represents a recent model of the dendritic array, including many of the necessary components and their functions required to produce its treadmilling dynamics. (d) At the scale of the whole cell, as seen in this example of a keratocyte, the front must assemble structures to protrude as the rear disassembles and contracts at the same net rate, demonstrating a treadmilling of the whole cell as it moves forward (top). The arrows represent the extent of protrusion and retraction needed in a model of keratocyte motility. Actin (blue) localized at the leading edge and myosin II (pink) at the contracting rear of a keratocyte fragment provide the necessary protrusion-contraction dynamics for movement (bottom). (e) In larger cells, interactions between the actin and microtubule cytoskeletons allow for coordination of assembly and disassembly dynamics over greater distances and longer times. The association of microtubules to the actin network positively regulates their polymerization at the front and induces their breakage through contraction forces at the transition zone of the lamellipodium. These physical effects on microtubule dynamics can activate the global signalers, Rac1 and RhoA, in different regions of the cell, and their regulation further enhances the cell’s treadmilling dynamics. (a) Reproduced and modified from (18) & #8211; by permission of The Royal Society of Chemistry and from (137), by copyright permission of The Rockefeller University Press. (b) Reproduced and modified with permission from (21), Copyright (2000) Blackwell Publishing. (c) Reproduced and modified with permission from (41), Copyright (2003) Nature Publishing Group, http://www.nature.com/. (d) Reproduced and modified with permission from (116), Copyright (1993) Nature Publishing Group, http://www.nature.com/ and from (61), Copyright (1999) with permission from Elsevier. (e) Reproduced and modified with permission from (62), Copyright (2003) Nature Publishing Group, http://www.nature.com/.
treadmilling actin filament requires further higher-order organization to leverage its basic unit of motility to function over greater distances and at greater speeds. Large complex actin structures have been observed in the protruding regions of cells (27, 28), and experiments show that treadmilling of this actin meshwork as a whole occurs during cellular motility (29–31). Ten years ago it was clear that molecular mechanisms must exist to allow for the regulated creation of nucleation sites at the cell periphery, desequestration of monomeric actin, and regulated depolymerization at the other end of the treadmilling meshwork (32). Tremendous progress has been made in identifying specific molecules involved in this process and the mechanisms by which they contribute to what is now known as “dendritic array treadmilling” (33). The coordinated effort of these proteins has been extensively reviewed (34–41).

The orientation of actin’s assembling end, causing protrusion at the front, and its disassembling end at the back dictate the overall polarity of the dendritic array. The nucleotide hydrolysis and polymerization properties of an actin filament set up the spatial and temporal regulation of the array’s behavior by modulating the association of various actin binding proteins that feedback on actin polymer dynamics. The speed at which a filament can polymerize or depolymerize, which is dependent on the surrounding components in its environment, and rates at which nucleotide hydrolysis occurs throughout the filament contribute to the overall dynamics of the array. The different conformations of ATP- and ADP-bound actin subunits within the filament control where accessory proteins bind preferentially and exert their function. The interactions between actin and its binding proteins leverage and amplify the temporal regulation intrinsically provided by the actin filament to provide a spatial framework for the dynamics of the dendritic array. The cell sets up the intrinsic outward polarity of the entire dendritic array by localizing necessary regulatory proteins to the spatial boundary of its membrane.

The assembly and protrusion of the front end of the array is enhanced by the Arp2/3 complex, which can nucleate branched actin filaments off the sides of other filaments, thereby increasing the availability of actin barbed ends for enhanced polymerization (42, 43). This activity is itself spatially controlled because there seems to be a preferential association of Arp2/3 with regions of actin filaments near the new barbed ends (44) and may be due to its preference for ATP-actin within the filament [reviewed in (37)]. Another spatial restriction to Arp2/3 complex activity is that the complex must be activated by an accessory protein, such as a member of the WASP family. These accessory proteins are in turn regulated by coactivators, such as phosphatidylinositol 4,5 bisphosphate (PIP2) and CDC42, which are anchored to the membrane. In addition, actin branches can dissociate over time, upon which free actin filament pointed ends can anneal to other filaments deeper within the array. This debranching may be spatiotemporally regulated as well by phosphate release upon nucleotide hydrolysis on actin [reviewed in (38)]. A further layer of temporal regulation is possible owing to the capability of the Arp2/3 complex itself to hydrolyze ATP,
although the role for this activity in regulating array dynamics is not yet clear (45, 46).

Some accessory proteins contribute to an increase in the number of free barbed ends through the severing of actin filaments, which include ADF/cofilin (34–41, 44) and gelsolin (34–41, 47). The number of available barbed ends is negatively regulated by barbed end-capping proteins, which can also prevent annealing of filaments but are in turn regulated by tropomyosin; this allows for filament annealing in their presence (38). In addition, tropomyosin protects filaments from severing, and it is spatially regulated, resulting in its absence from the leading edge and presence deeper in the lamellipodium (48). Such dual roles are also apparent for some other proteins. One is ADF/cofilin, which, in addition to severing filaments, increases the rate of depolymerization in order to speed the recycling of monomeric actin (49). Regulation of the degree of depolymerization can also occur through pointed end capping, via Tmod3, an isoform of tropomodulin (50). The overall depolymerization activity must be spatially regulated in order to ensure recycling of monomeric actin to the front end of the array. This regulation is demonstrated by the localization of activated cofilin to the lamellipodium in fibroblasts (51) and by the more localized absence of cofilin along the periphery of the leading edge in rapidly moving keratocytes (33). Recycling of ATP-actin to the leading edge occurs through sequestration of free monomers, via thymosin β4 and profilin, to control their concentration and promote nucleotide exchange (52). The preferential affinity of profilin for ATP-actin and spatially localized binding partners such as vasodilator-stimulated phosphoprotein (VASP) then allows profilin to act as a shuttle and increase the availability of polymerizable actin to the front of the array. Figure 2c shows a current model for many of the molecular components and their mechanisms.

Multicomponent combinatorial regulation of the actin polymerization dynamics creates a higher-level structure that self-organizes and continuously recycles the necessary molecular components to allow the array to treadmill forward. Polymerization against a load can itself produce a force [reviewed in (21)], and overall polymerization of the dendritic array is a mechanism by which protrusive force is generated at the leading edge of moving cells (31, 53). Although crawling cells have more complex requirements for their movement (see sections below), some intracellular pathogens, such as *Listeria monocytogenes* and *Shigella flexneri*, move solely by harnessing the dendritic array geometry to their surface through their own regulatory proteins, ActA and IcsA, respectively [reviewed in (54)]. Interestingly, even though *Listeria* and *Shigella* motility could be reconstituted with a subset of the proteins present in the array, a crowding agent was required (55), demonstrating an essential role for the crowded nature of the cell in regulating the dynamics of the array to produce movement. Persistent motion can be simulated by a simplified mathematical model through a section of a cell incorporating barbed end polymerization, branching near barbed ends, pointed end depolymerization, debranching, and de novo nucleation to ensure maintenance of free actin (56). Although the direction
of this motion can switch through stochastic fluctuations in model behavior, it remains correlated with the direction in which the barbed ends point. In addition, the model creates an asymmetric filamentous actin distribution, as is seen in lamellipodia (57) [reviewed in (38)]. Long simulations of this model produce an imitation of the persistent random walk observed for crawling cells. Thus, the basic underlying spatial polarity the cell uses to create a treadmilling array for protrusion can in fact produce directed motion. While the dendritic array can span a large protruding region of the moving cell, some of its protein components must be rapidly turned over spatially to support its fast-paced dynamics, leading to a problem of delivery of components to the leading edge, which is considered later.

**Coupling the Actin Array to Cell Contraction and the Adhesion/De-adhesion Cycle**

When the dendritic array is assembled inside a membrane-bound cell, the protrusion it causes will not, by itself, allow the cell to crawl. A section of the cell will protrude because of polymerization dynamics but will not suffice for the whole cell to move unless the rear is able to retract, effectively allowing the cell to treadmill (Figure 2d). The mechanism of retraction is thought to be dependent on contraction of actin fibers via myosin II (58, 59). In keratocytes, myosin II thick filaments are colocalized to regions in the cell body and transition zone where actin filaments are in bundles or asters, increasing in concentration as the actin filament concentration decreases (57). This spatial regulation suggests a role for the contracting functions of myosin II in the retracting rear. Fragments of keratocyte lamellipodia can polarize and move persistently (60), showing that the combination of the dendritic array treadmilling at the front and myosin II-mediated contraction at the rear is sufficient for their movement (Figure 2d) (61).

In cells much larger than the specialized, fast-moving keratocytes, these same principles hold, but additional levels of spatial and temporal regulation are required to coordinate the necessary protrusive and retractive elements of treadmilling over much larger distances. Well-regulated signaling cascades can allow for large regions of the cell to behave in a unified manner and transduce spatial information globally. The cell links the dynamics of the actin meshwork to that of the microtubule cytoskeleton, which due to its thicker and stiffer filaments can provide structural regulation over greater distances (Figure 2e). The interactions between the actin and microtubule cytoskeleton coupled to reinforcing signaling cascades set up a polymerization/contraction treadmill [reviewed in (62)]. In this treadmill, the polymerization activity of the microtubules at the protruding region activates the global signaler, Rac1, which in turn stimulates the polymerization of both actin and microtubules. As the microtubules become displaced toward the contracting region of the cell, they are broken through its compressive forces. The catastrophic shortening of microtubules in the contracting region activates another broad-acting signaler, RhoA, which stabilizes microtubules and stimulates the contractile actin structures (62). Thus the microtubule network is coupled to the actin-based protrusion/retraction organization, linking
structural spatial information contained in the dynamic behavior to temporally regulated signaling molecules to coordinate polymerization and depolymerization over greater distances (Figure 2e).

The cell translocates across a solid substrate through a treadmilling process in which protrusion is coordinated with increased adhesion in the front and retraction with loss of adhesion in the rear, effectively requiring a treadmilling of adhesions. Many different cellular molecules interact with the different substrates on which the cell crawls, and they are regulated through signals and mechanosensory processes to allow for their necessary, rapid turnover [reviewed in (63)]. For efficient movement, the cell must have strong attachments to the surface in the protruding regions, as seen in keratocytes (64, 65). The action of myosin II in the rear pulls at the adhesions and weakens them, allowing for the cell body to be pulled toward the tightly adhering front. The localization of adhesions in these specialized cells is optimally coordinated with protrusion and retraction to allow for rapid movement. In larger cells, microtubules can negatively regulate focal adhesion when targeted to and closely associated with these sites (66). The cell thus engineers a direct link between protrusion and adhesion dynamics, regulated through the polarity of the cellular cytoskeleton, to provide the necessary coordination to move.

An impressive example of how the whole cell treadmills along a substrate is the nematode sperm cell [reviewed in (67)]. This cell contains very little actin, but it uses a completely different polymerizable protein to generate its movement. This major sperm protein (MSP) can polymerize into helical filaments and, because of its own specific shape and interactions, can further assemble into higher-order structures. MSP assembly is spatially regulated by a membrane-bound activator, MSP polymerization organizing protein (MPOP), which is only activated at sites where MSP polymerizes (68). The MSP polymerized assemblies consist of multiple branched and packed fiber complexes that span the protruding lamellipodium and assemble at the front end while disassembling at the rear. The protrusion and adhesion dynamics are both mediated in time and space by mechanisms that are pH-sensitive and can therefore be coordinated by the local environment. Two recent physical models have integrated information on the dynamics of the Ascaris sperm cell and the mechanics of the polymerized MSP gel (67, 69). These models can recapitulate the major features of crawling movement and have identified new possible experimental directions to further elucidate the mechanism by which cells treadmill forward by hierarchial temporal and spatial enhancement of the inherent asymmetry of protein self-association.

REGULATING THE HIERARCHY OF DYNAMIC ACTIN ORGANIZATIONAL STRUCTURES

The neutrophil, keratocyte, and growth cone all use similar molecular machinery to achieve net actin filament growth at the front and loss at the rear, but they manage to achieve such varied dynamic morphologies in their movement through
the specific regulation of their functions and interactions in space and time. Any given cell must therefore be able to reorganize many of its different types of subcellular structures to provide the higher-level reorganization it needs to respond to a specific set of conditions, such as activation of signaling molecules upon exposure to a chemoattractant in a neutrophil. The intracellular local environment is full of random movement from thermal energy, and amplification of this movement results in constant fluctuations over variable size and time-scales. The cell must be able to create order out of these fluctuations and the transient interactions of its components and regulate their functions in space and time. The uniqueness of such dissipative self-organizing systems is that they must experience a constant flow of energy and matter to persist, and they have to minimize the rate of energy loss to reach their dynamic steady state (70, 71).

These systems exploit the stochastic nature of the environment to create a dynamic, ordered organization by amplifying random fluctuations. If an environment is near a critical set of conditions that allow multiple outcomes, the stochastic dynamics of these conditions can determine the resulting behavior. Thus the ever-changing transient dynamics within the cell create the ability for a behavior or organized structure to form. Because of the finite number of molecules within the cell and the stochastic nature of their exact locations, even when targeted to a specific region of the cell, the relative ratios of necessary components can precisely regulate their ability to interact and self-organize into different higher-order functional structures. Once an organized steady state is reached, the dynamic functional unit has to persist until another is needed, meaning that the system must be less sensitive to certain components than to others that the cell perceives as important environmental signals, such as chemoattractant and repulsive agents. Good examples of self-organized units with differential sensitivities are the dynamic, self-organized structures created by microtubules and their motors in vitro (72). These structures were shown to be sensitive to the relative ratio of motors to microtubules, forming asters only after a motor concentration threshold was reached. A computational model of this process showed that the extent of aster formation was highly dependent on the speed of the motor along the microtubule, while changing the affinity of a fast-moving motor for the microtubule had less of an effect (72). Such results would ensure the formation and persistence of these robust dynamic structures in the presence of distinct motors with similar speeds.

Biochemical patterns have been shown to form if there is a local self-enhancing activity at balance with a more global inhibition [reviewed in (73)]. The amplification must be nonlinear in nature (73), for example by being autocatalytic through cooperativity or positive feedback, in order for the amplified behavior to dominate among surrounding fluctuations and persist in the presence of the global inhibitor. In the context of cell motility, patterns constitute dynamic, self-organized structures regulated in space and time, such as overall cell polarity. An example is when neutrophil-like HL60 cells respond to a chemoattractant by activating two distinct G proteins regulating two separate
signaling pathways with different locations in the cell, one acting at the front and one at the rear, establishing cellular polarity (74). The pathway in the front activates positive feedback between Rac signaling and actin polymerization, amplifying protrusion, while the signal in the rear activates Rho signaling and promotion of actin-myosin contractile structures, inducing retraction. Polymerization and contraction are regulated in spatially distinct regions of the cell and inhibit each other, strengthening the amplification of the signal-induced process leading to large-scale morphological changes in the front and the rear (74). Here the self-organization of two functioning dynamic substructures spatially regulated within the cell requires amplification of a set of signals that feed back into reorganizing these structures to a point where they are at steady state and can exert their functions, allowing the cell to move.

The cell maintains its sensitivity by regulating the self-organization of intracellular structures through their own sensitivity to the ratios of their components. A molecule with a certain function can cause different outcomes based on its concentration relative to its binding partner (see α-actinin in a section below), or a multifunctional molecule can have different functions that become dominant based on the local ratios of the components (see VASP in a later section). The robustness of an outcome created by the sensitive self-organized structure can be regulated through relative insensitivity to concentrations of specific components (72) or through redundancy in molecules able to provide a specific function (see examples below). The next section explores specific examples of structures that are sensitive to the balance of their environment and examples of mechanisms to ensure robustness of necessary functions through redundancy in molecular components at all levels of cell motility. Their implications are considered within the context of subcellular self-organizing structures that are spatiotemporally regulated by the cell to produce motion.

**Intrinsic Properties of Actin Contributing to Its Organization**

There is more to the life of actin filaments than simply polymerization and depolymerization in coordination with nucleotide hydrolysis. Structures forming within filaments immediately upon polymerization can be different from those found later. This difference in structure could be related to nucleotide hydrolysis because filaments show different conformations of actin in the presence of ATP, ADP, or a transition state analog (75, 76). Additionally, various substructures are possible within a single actin filament. Actin subunits have been shown to be capable of a tilted conformation in some regions of a filament in the presence of unbound ADF/cofilin, thus changing the available interaction sites to binding partners along the filament under these conditions (77). Cross-linking studies have identified short-lived actin dimers that are thought to have various associative properties and eventually change conformation to that of the stable long-lived associations found within the filament [reviewed in (78)]. This special conformation can be positively regulated by high concentrations and specific
types of divalent or multivalent cations. Filaments comprised of dimers interacting in this way are rough in appearance, and they may function as cross-linkers between antiparallel actin filaments or cause branching of filaments through their special geometry (78). The fact that this orientation of two actin monomers is short-lived and may induce an inherent branching capability illustrates a way that the associative interactions between actin itself influences the timing of the actin polymer structure and the subsequent spatial restrictions for interacting proteins. At higher divalent cation concentrations, other higher-order polymeric actin structures can be seen such as “two-layer rafts” in which shielding by the ions allows actin filaments to self-associate (79), and in the presence of other polycations, actin bundles are formed (80). In addition, experiments and models have shown that, within a crowded solution, actin filaments spontaneously separate into bundles (81), which has implications for the crowded environment of the cytoplasm. Specific differential conformations within actin filaments as well as higher-order associations in the absence of other binding proteins are dependent on the components in the environment and affect the actin structures that form and their mechanical properties.

In vitro purified actin polymerizes within minutes and forms a filamentous network, which over hours acquires the stiffer elastic properties of a gel (82). The rate at which gelation of the network occurs is not due to polymerization itself but rather homogenization of the network through redistribution of actin from regions of higher density to lower via its reptation (82). Recent microrheological experiments on living cells suggest that the mechanical properties of this gel may be those of a soft glassy material near its metastable glass transition, able to regulate its properties through changes in the dynamics of the cytoskeletal meshwork (83). Thus the global mechanical properties of actin meshworks are sensitive to local densities therein. The viscosity due to actin in the Dictyostelium cytoplasm can be regulated by the osmolarity of its environment (15). The relationship between this viscosity and cellular volume is nonlinear, indicating that the mechanical state of the actin meshwork within the cell is variable and sensitive to the volume. Given the nonlinear dependency of protein associations on the crowding of the environment (11), the mechanical properties of the actin meshwork may well be very sensitive to the volume of its environment. The examples in this section show that some specific higher-level organizations of actin networks are energetically preferred. In order to leverage these energetic advantages into rapid cell shape changes, the cell stabilizes and regulates the different structures found in organized actin networks through dynamic interactions with accessory proteins.

Sensitivity and Robustness in Multicomponent Subcellular Actin Structures

Although actin itself can spontaneously form higher-level assemblies, the cell regulates the spatial and temporal distribution of its different dynamic actin organizations through accessory actin binding proteins. These proteins can stabilize structurally and functionally distinct organizations such as actin gels or
actin bundles (Figure 3a), which are sensitive to the relative concentrations of their protein components. In vitro the addition of α-actinin to actin induces a threshold concentration-dependent transition from a gel-like to a bundle-like organization that can be predicted by a simple model taking into account relative concentrations and affinities of different α-actinin proteins (84). Adding a spatial boundary condition, by introducing actin and different binding proteins into vesicles, induces phase transitions in the organizational structure of the multi-protein actin networks dependent on the size of the spatial constraint, relative concentrations of the components, and temperature (85). These transitions can be spatiotemporally regulated within a cell through the localizations of binding partners to specific structures, such as fimbrin to filopodia or α-actinin to stress fibers (86). Robustness of these structures is achieved through the high numbers of actin binding proteins in the cell, many with overlapping functions. For example, different isoforms of myosin in Dictyostelium can compensate for each other functionally when one or several are removed from the cell (87).

Whereas certain actin binding proteins cause specific structural reorganizations of the network based on their topology of interaction with actin, others can have multiple more complicated functions, which affect both spatial and temporal regulation of motility. Consider the Enabled/VASP (Ena/VASP) family of proteins. These proteins have been shown to interact with F-actin, have high affinities for profilin (thus recruit monomeric actin for polymerization), have actin nucleation capabilities independent of Arp2/3, have anticapping function, and can dramatically affect the organizational state of actin meshworks [reviewed in (88)]. They localize to tips of filopodia, focal contacts, and the leading edge of protruding lamellipodia, and their function is redundantly ensured because there are multiple members of this family that can compensate if one is deleted from a cell (88). In addition, the various functions of each of these members may have slightly different effects on the resulting dynamics these proteins control. Changing the concentrations of Ena/VASP relative to other proteins at the leading edge creates dramatic changes in the architecture of the filament network. An increase leads to elongated filaments, while a decrease results in a more highly branched organization (89), demonstrating the sensitivity of the environment to the balance of Ena/VASP’s different functions.

Protrusion dynamics at the leading edge can be sensitive to other components as well. The Arp2/3 complex, in conjunction with its activating cofactors, creates a dendritic branched network. Its spatial organization, the angle between mother and daughter branches induced by the complex, may be optimized for cellular protrusion (90). Temporal regulation of Arp2/3 occurs through the combinatorial control of its carefully regulated activators and additional cofactors. An example of this is cortactin, which can stimulate Arp2/3 actin nucleation by itself as well as function synergistically with N-WASP (91) for further stimulation of nucleation. Cortactin interacts with its cofactor dynamin, a protein involved in endocytosis (92) that can effect Arp2/3-cortactin–dependent actin nucleation in a concentration-dependent manner. Dynamin stimulates nucleation at lower
**In vitro**

a. Actin with branching protein

Actin with bundling protein

b. Pseudopodium  Lamellipodium  Filopodium

c. Leukocyte  Keratocyte  Growth cone
concentrations and inhibits at higher (93), thus regulating the effect of cortactin on actin nucleation by making nucleation sensitive to levels of other components in the local environment. The cell provides many such tuneable parameters, carefully and continuously regulating actin nucleation.

Just as the dynamics of actin nucleation in the dendritic network must be carefully regulated in space and time to control protrusion, nucleation must also be regulated in creating other types of actin organizational structures with nonprotrusive functions. Another family of proteins, the formins, has been identified with the ability to nucleate actin polymerization directly, and formin dynamics are sensitive to other proteins in the environment, such as profilin (94). These proteins are implicated in nucleating filamentous actin structures, such as stress fibers, yeast actin cables, and contractile actin bundles, needed for cytokinesis [reviewed in (94)]. In addition to providing a different nucleator for filamentous structures, the cell also inhibits Arp2/3 branched nucleation through proteins, such as eplin (95), that can stabilize bundled actin structures through spatial regulation of their activity. The complex multicomponent regulation of formin activation, its actin nucleation, and its stabilization of induced structures by

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**Figure 3** The various organizational structures produced by actin and its interacting accessory proteins can create distinct morphologies of movement. (a) In vitro experiments visualizing the structure of the actin filament meshwork in the presence of an actin cross-linking protein. Actin with ABP forms a gel-like organization (top), and actin with the Abl-related kinase, Arg, forms bundles (bottom). (b) Within a cell’s protruding region, actin structures depend on the relative concentrations of the components in their environment. Ultrastructure of the tangled actin meshwork in a *D. discoideum* pseudopod (left), the branched dendritic array in a keratocyte lamellipodium (middle), and a bundled filopodium from the leading edge of a B16F1 mouse melanoma cell (right) are shown. The structural organization in the pseudopod is more disorganized and tangled, and it contains branches and long filaments throughout. The dendritic array in the lamellipodium is more ordered and displays more free ends. The filopodium emerges from a lamellar meshwork and is made of tightly bundled actin filaments, held together at the tip by a “filopodial tip complex.” (c) SEM images of three cells with different morphologies in their protruding regions and movement are shown. The first is a leukocyte, ~15 μm long, with its pseudopod, labeled P (left). Next is a gliding keratocyte, ~28 μm in diameter, with its thin lamellipodium extending rightward in the direction of motion (middle). The last is the growth cone of a chick dorsal root ganglia (DRG) neuron with its many extending dynamic filopodia (right); the cell body is ~15 μm in diameter at its widest. (a) Reproduced and modified from (138) by copyright permission of The Rockefeller University Press and with permission from (139), Copyright (2001) National Academy of Sciences, U.S.A. (b) Reproduced and modified from (140), (57), and (97) by copyright permission of The Rockefeller University Press. (c) Reproduced and modified from (141) by permission of Oxford University Press, with permission from J. Lee, and from (142), Copyright (1985 Journal of Neuroscience Research), reprinted by permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.
inhibiting the other, branched, nucleation pathway indicate once again that activation of actin nucleation is carefully regulated and sensitive to the identity of proteins, their activity state, and their concentrations. These examples demonstrate the cell’s combinatorial control over actin network structures, which are sensitive to the local environment. The complex spatiotemporal regulation of the effector components results in subcellular dynamic structures with distinct organizations.

Transitions Between Higher-Level Organizational Actin Structures

For dynamic cell motility, it is not sufficient that the cell be able to build various types of higher-order actin structures, it must also be able to switch among them. To extrapolate the biochemical observations described above to a cellular level, local spatial constraints and ratios of actin to its binding partners can sensitively effect the higher-level organizations of actin networks. The cell must be able to regulate the creation of branched and bundled actin networks, which it does through differential regulation of the nucleation of such structures and through the reorganization of one structure into the other. The transition between branched and bundled networks made of flexible polymers lies at a critical metastable boundary (96). Theoretical calculations show that small changes in the density of cross-linker can induce a phase change from one type of structure to the other. Thus the transition between actin bundles and branched structures occurs through changes in the relative levels of components in a specific region of the cell. Because actin filaments in a living cell turn over so rapidly, with average half-lives on the order of one minute, slight changes in component availability can cause large-scale transitions across phase boundaries even if the phase transition would normally be kinetically forbidden. This concept is beautifully demonstrated in recent work on the transition between lamellipodial and filopodial actin organization (Figure 3b) at the leading edge of cells and in an in vitro assay (97, 98). Filopodia can form through λ-precursors in which the dendritic organization of the leading edge allows for filaments to elongate, associate at their tips, and form bundles. Specific proteins, such as VASP, may regulate filopodial initiation by its anticapping function and allow bundles to form at the cell edge through organizing a tip complex. The filopodial bundles are subsequently stabilized by the presence of other actin bundling proteins, which include fascin throughout the whole filopodia and α-actinin in regions closer to the cell (97). Further, specific factors that may control the transition between the lamellipodial and filopodial organization were identified. In an in vitro assay, filopodial structures could be induced from actin clouds around beads coated with Arp2/3 activator through changing the concentrations of components in the environment. These structures were shown to be sensitive to the identity of the nucleator and to the concentration of capping protein (98). Thus the balance between the relative strengths of the nucleator and other necessary components, such as capping protein, and their resulting functions in the local environment could induce a large-scale reorganization of dynamic self-organizing actin
structures. Such a global reorganization of intracellular structure is seen over brief time intervals in the activation of platelets during which an increase in intracellular calcium first activates gelsolin and then PIP$_2$, leading to a dramatic change of the actin meshwork, with the extension of lamellipodial and filopodial structures as the platelet flattens (99). Within a cell, such transitions are spatiotemporally regulated through sensitive responses to the local environment and more globally regulate the types of protrusive movement created.

To control the different actin organizations within the leading edge of the cell, global signaling molecules can induce one structure or another, as seen in the capability of Rac1 to induce lamellar ruffling and of RhoA to induce stress fibers in serum-starved 3T3 cells [reviewed in (100)]. In order to ensure that a cell can elicit its specific needed response, multiple protein interaction pathways can lead to the same effect and within their delicate mutual cross talk can add layers of regulation onto the final behavior. An example is seen in the regulation of filopodial and lamellipodial formation through interactions of various sets of proteins. CDC42 can directly interact with WASP and thereby regulate the nucleating activity of Arp2/3 [reviewed in (101)]. However, another interacting pathway among CDC42, IrsP53, and Ena/VASP proteins demonstrates an Arp2/3-independent mechanism of regulation [reviewed in (36)]. Recently the discovery of IrsP53 colocalizing with WAVE in lamellipodia and filopodia, independently of Ena/VASP proteins, has demonstrated an additional pathway through which the cell regulates the protrusion dynamics at its leading edge (102). Because the spatial and temporal regulation of protrusive actin structures can be a vital response by a cell to its environment, it provides multiple, redundant pathways to ensure the behavioral response required, such as the neutrophil’s ability to continuously follow its microbial prey.

The fact that a cell has many different individual protein components that are able to provide similar functions is clear from considering intracellular pathogens. _Listeria_ and _Shigella_ both move intracellularly through the same mechanism, propelling themselves by usurping the cell’s dendritic array organization. They do so by entering the cell’s regulatory pathway at different places; _Listeria_ provides its own Arp2/3 activator, and _Shigella_ recruits N-WASP to activate Arp2/3. Recently, _Burkholderia pseudomallei_ was shown to recruit Arp2/3 to its surface to create actin tails. Neither N-WASP nor Ena/VASP proteins were needed (103), indicating another mechanism by which an intracellular pathogen can hijack the cell’s machinery. This demonstrates how different regulators can produce similar structures and how the same regulators can create different higher-order structures in varied environmental contexts.

**COORDINATION OF DIFFERENT PROPERTIES OF A CELL TO PRODUCE MOVEMENT**

For a cell to be motile, the balance between different protrusive organizations and their regulation must be well coordinated. Recall the example of the three different cells with distinct dynamic morphology moving through very dissimilar
environments (Figure 3c). Despite their varied geometries, a lymphocyte crawls through three-dimensional tissues just as fast as a keratocyte on a two-dimensional surface, each moving in a way optimized for its environment (1, 60, 104). To achieve this rapid movement the cell must globally coordinate its behavior with the rest of its components in space and time as well as its environment to span the relatively large distances and long times required for whole-cell motility.

Coordination Within Dynamic Actin Structures for Cellular Motility

To consider how a cell coordinates its protrusive actin structures, it is useful to consider a simplified system in which a bead is uniformly coated with a nucleator (such as *Listeria* ActA, which activates Arp2/3) and is placed into cytoplasmic extract (104a). The nucleator recruits from its surroundings the necessary components that then self-organize into a dynamical structure that propels the bead using the same molecular mechanisms that allow a cell to extend a protrusive actin structure (see array treadmilling above). Stochastic fluctuations in the dynamics of the actin cloud that forms around an ActA-coated bead allow it to enhance and amplify local asymmetries that are then self-organized into an actin “comet tail” to move at steady state (105). The behavior of all the needed molecular components is inherently coordinated to form a dynamic protrusive structure similar to that seen inside cells. Cells must also coordinate signaling molecules to regulate these dynamics. Similar stochastic fluctuations in peripheral localization of activated CDC42 in yeast cells allow for amplification of actin nucleation and for the production of polarized CDC42 caps along a region of the cell periphery through a positive feedback loop (106).

While amplification of stochastic fluctuations can intrinsically induce a cell or cellular region to locally coordinate the interactions between its molecules to produce a protrusive behavior, this can also occur in response to an external signal. Neutrophils respond to the local presence of a chemoattractant by inducing polymerization toward this environmental signal and coordinating the formation of nucleation sites in order to amplify the protrusive behavior (107). The whole cell is then able to move in the direction of the chemoattractant. Likewise, stimulation of fibroblasts with platelet-derived growth factor induces global remodeling of the cytoskeleton. These dramatic changes may occur partly through the coordination of multiple proteins, which include actin, dynamin, cortactin, Arp2/3, gelsolin, and N-WASP, to form dynamic circular waves that move through the cell upon stimulation (108). These waves affect cortical actin structures, disassemble stress fibers, and are required for lamellipodial protrusion over large areas. Although they seem to occur only once upon stimulation (108), similar-looking waves of actin can occur in stationary, unstimulated B16F1 melanoma cells, MDCK cells, and in *Dictyostelium* [(109, 110) and S.M. Rafelski, unpublished observation] and have been observed to create protrusive structures at cell boundaries (110). Thus large-scale dynamic organizations...
within the cell allow for coordination of a new state on a more global scale, for example the transition of a resting to a moving cell, through the coordination of many of the regulatory proteins to cover the large distances over which this remodeling must occur.

Coordination Between Physical and Chemical Processes

A cell experiences physical forces from its environment and from some of the biochemical processes occurring intracellularly, such as actin polymerization and myosin-dependent contraction. It must therefore coordinate the interactions between the biochemical molecular processes and the physical components in order to respond to its environment effectively. This section focuses on the interplay of interactions between physical and chemical processes at all levels within a cell. On a small scale, experiments have shown that the specific spatial distribution of an actin nucleator, ActA, on the surface of small (3 μm) vesicles not only allows for these vesicles to move by actin polymerization, but it also causes a partitioning of the forces generated during this process (111, 112). The combined forces compress the vesicle, and modeling of their distributions and magnitudes show that while there are many protrusive forces along the sides, there are strong retarding forces at the rear of the vesicle. ActA has nucleation activity via activation of Arp2/3 (113), causing protrusive force, and interacts with the comet tail via Ena/VASP proteins as well as Arp2/3 binding (114, 115), contributing a retarding force. These two types of forces partition into local subregions through the spatial polar distribution of ActA’s biochemical activities along the vesicle surface, and they contribute to the physical forces and dynamics produced by the polymerizing actin comet tail. Taking a more global viewpoint, if one considers the general forces on the scale of a whole keratocyte that are produced in order for it to move (Figure 2d), they too are seen to be spatially partitioned. Protruding forces exist at the front, disappear at a transitional zone, and become retracting forces at the rear (116, 117). The cell as a whole, therefore, organizes its local behaviors to partition its forces globally for movement.

As is the case with many processes in the cell, causes and effects can become interchanged owing to complicated feedback loops. Physical changes in the environment can regulate biochemical pathways and the subsequent local and global reorganizations they produce. For example, locally produced forces may induce greater curvature at those regions of the cell membrane. Transient changes in membrane curvature induced by addition of phospholipid to platelets have been shown to cause filopodia to form (118). The change in curvature locally activates phosphoinositide 3-kinase (PI 3-kinase), which in turn activates multiple molecules, including PIP$_2$, known to be involved in modulating actin dynamics [reviewed in (119)]. This effect was also seen in fibroblasts in the absence of microtubules, giving a less well-defined cellular structure (118). In this example, a local change in the physical environment triggers signals implicated in regulating protrusive actin structures to reorganize the protrusion dynamics locally. To restrict the reorganization to a specific region, other
structural organizations, such as the microtubule network, can regulate the extent of the effect these changes have on the cell.

As was discussed above, a cell needs to create and regulate traction to move across a substrate. The attachments to the surface need to be spatially well distributed to have a positive effect on the movement and must be short-lived not to arrest the cell completely. An example of precise regulation was directly shown through the transient interactions of Arp2/3 and vinculin at new sites of adhesion (120). Activators of Arp2/3 and vinculin stimulated their interaction, and cells expressing mutant vinculin unable to associate with the Arp2/3 complex showed decreased lamellipodial protrusion (120). These experiments demonstrate the direct link is transient in nature and allow polymerization and adhesion to be spatially coordinated between active Arp2/3 complex and active adhesion sites. In addition, the forces placed on initial sites of adhesion can regulate their development into more adhesive structures (121) and therefore their capability to coordinate with the rest of the cell for its migration. Locally exerted forces, such as pulling on a macrophage adhering to a bead, can regulate the polymerization dynamics, stimulating the formation of protrusive actin structures as seen when a macrophage engulfs a pathogen (122). Stretching forces occurring when a cell’s protrusive rate is greater than its retractive rate can regulate calcium release in keratocytes through stretch-activated calcium channels (123). The resultant increase in intracellular calcium levels stimulates the retraction of the cell rear to restore the net steady-state balance between protrusion and retraction rates. Thus the physical forces exerted on the cell as it moves communicate with its intracellular molecule-based dynamic reorganizations to coordinate its movement.

Cellular coordination also occurs through global effects of volume changes and their mechanical consequences on the actin network. The spatial distribution of ion channels is regulated such that their effect at the leading edge of crawling cells induces water uptake and volume shrinkage at the contracting rear (124). Additionally, cells can exhibit dramatic actin flow, as seen in Aplysia growth cones, in the direction opposing motion (30). The hydrodynamic environment within the cell is therefore rapidly changing. Moving Dictyostelium cells display much greater diffusion of intracellular GFP than do stationary ones, making their effective viscosity 1.4 times lower when moving while not changing their volume significantly (15). In addition, diffusion is slightly enhanced in the protruding region of the cell, indicating local differences in the mechanical properties of the dynamically polymerizing actin structures. Experiments examining actin dynamics during cell protrusion show a rapid trafficking of monomeric actin to protrusive structures in fibroblasts faster than can be accounted for by diffusion (125). Other experiments investigating the relative distributions of 10 and 70 kDa dextrans in cytotoxic T-lymphocytes reveal a greater accessibility of the larger dextran to regions of cellular protrusion upon recognition of target cells (126), contrary to the predictions of simple diffusion or molecular sieving. Flow-like properties of the liquid phase of the intracellular environment may allow
molecules moving through their crowded environment within actively polymerizing regions of a cell to exhibit dramatic mobility differences. In an extreme case, the sea cucumber Thyone briareus extends its long 90 μm acrosomal process fully within ten seconds through polymerization of actin, a process requiring an active flow-dependent transport of monomer to the tip of the extending process (127). Actin meshworks have gel-like mechanical properties, and it has been suggested that hydrodynamic pressure on gels may create channels (125). These hypothetical channels could provide a route for larger molecules or complexes to cover their required distances on shorter timescales simply through the inherent mechanical properties produced by dynamic polymerizing structures acting in coordination with cellular movement. This type of rapid transport directed to the leading edge of protruding regions has been previously considered in the context of myosin II-dependent contractions at the rear of the cell, providing the necessary intracellular flow (128). Further work is needed to understand this hydrodynamic flow mechanism and the positive effect its coordination throughout the cell could have on continuous protrusion dynamics.

Coordination Between Local Behaviors and Global Responses

For a cell to move and respond to its environment, a local behavior induced by an environmental signal clearly must be coordinated and coregulated with a global response to the extent required by the cell. Growth cones explore their environment and, upon discovering chemical signals, must change their direction of movement to follow their required path and stabilize microtubule bundles in the axons of their neurons (129, 130). Local changes in substrate adhesion on growth cones have been shown to interfere with their continuing retrograde flow and can induce large-scale cellular reorganization of the actin and microtubule network [reviewed in (131)]. Additionally, disruption of actin bundles at the periphery induces a collapse of a region of the protrusive structures nearby, leading to repulsive turning motion (132). Thus, a local cue can induce global reorganization within the cell, which results in a new large-scale behavior directly linked to the localized effect.

Now consider specific examples of regulated proteins affecting local protrusion dynamics and therefore the overall global response of the cell. The local activation of thymosin β4 in a keratocyte induces a local depolymerization of actin structures and a global response manifested in the whole cell, which pivots around that region through its coordinated protrusion-retraction dynamics (133). The multiple functions of the Ena/VASP proteins have been shown to have significant effects on the behavior of the dendritic array and of moving cells. The direct effect of Ena/VASP proteins on the dendritic array dynamics alone is exemplified in their strong positive effect on the rapid movement of pathogens, such as Listeria, for which dendritic array treadmilling is sufficient for movement. In Dictyostelium cells, DdVASP induces filopodial extensions and regu-
lates actin polymerization (134). It also localizes to the regions of the cell that respond to chemoattractant. In the absence of DdVASP, cells still extend pseudopods but no longer adhere well. The combination of these effects causes DdVASP null mutants to respond poorly to chemoattractant, effectively decreasing their directional motility (134). In neutrophils, sequestration of VASP proteins from the cell periphery causes a concentration-dependent decrease in migration rate upon stimulation with fMLP as well as a decrease in polymerization capability (135). Additionally, Jurkat T cells require Ena/VASP proteins for the rapid polymerization of actin extensions toward their target (136). These observations demonstrate a link between local Ena/VASP-dependent enhancement of polymerization, possible effects on adhesion, and the global effect this protein family can have on efficient chemotaxis, migration, or active actin dynamics in these cells.

In contrast to the previously mentioned cell types in which Ena/VASP proteins generally enhance cell movement, in unstimulated fibroblasts it has been shown that although Ena/VASP proteins induce local rapid protrusive behavior, evident as ruffling (89), their absence induces protrusion of a larger, more persistent lamellipodium and more rapid overall cellular movement. These apparently contradictory results may be reconciled by considering the biological context of motility in these cell types. Neutrophils and Dictyostelium cells are fast moving, responding to their environment in an active and directed fashion. The coordination between their local actin dynamics and global cellular movement is optimized for a quick response, as are the actin rearrangements in activated T cells. Unstimulated fibroblasts, however, move at rates 20 times lower than Listeria or neutrophils and may respond to cues in their environment, but they are not inherently motile cells dependent on rapid movement for their biological function. Perhaps the coordination between local protrusion and global motility is more complex or less efficiently regulated in these cells, leading them to benefit from a slower, more persistent protruding structure. Fibroblasts are also five times larger in diameter and must therefore coordinate their efforts over a greater distance, which may affect the cell’s capability to coordinate its behavior at a fast rate. Both a difference in the dynamics of the protrusive structures between these cell types and a possible difference in coordination efficiency caused by their varied sizes may explain the distinct effects of Ena/VASP proteins. These examples illustrate the complexity of cellular regulation of molecular, physical, and mechanical processes and the coordination between them to create motile behavior. They also demonstrate that the global outcome from the integration of local functions may be distinct in cells because of differences in their motile states, for example actively moving neutrophils in contrast to unstimulated fibroblasts. The effects that intracellular components can have on the dynamics of crawling cell motility should be considered within the context of the type and morphology of movements of various cell types as well as the functions that these cells perform in their supercellular environment.
STUDYING THE SCALES OF A CELL FROM MOLECULAR TO GLOBAL COORDINATION

The field is continuing to obtain specific detailed information on the identity, interactions, and dynamics of many molecules implicated in cellular motility and the ways in which a cell can integrate their functions, spanning the scales of proteins and molecular machines to the physical, mechanical, and dynamic properties of whole cells. In the continual attempt to understand the process by which a cell crawls, it is important to investigate the required interactions by perturbing cellular organizations at all of these levels. Biochemical experiments provide information on the identity of necessary components and on the dynamics of their interactions. Reconstituted systems of a limited number of proteins lead to an understanding of the specific capabilities of that subset of proteins in organizing structures and coregulating their functions. Experiments at the level of biochemical complexity of all molecular components of a cell, such as those using extracts, are needed to understand the basic principles of processes, without spatial and temporal cell boundary conditions, at an intermediate level of organization. Finally, direct investigation of the cell as a whole provides information on its overall temporal and spatial regulation as well as its physical and mechanical properties. Experiments at all of these levels are useful in providing frameworks for mathematical models of various cellular processes, which in turn can direct specific areas of further experiment and subsequently lead to more refined and accurate models. The integration of information learned from all levels of experiment must then be applied to the whole cell, to understand how it has been able to regulate the behavior of its components all along, from the individual protein through four or more orders of magnitude in space and time to the coordinated behavior of its movement. Likewise, additional investigation in the field can build on a mechanically sound, preexisting structure to coordinate biological, physical, and chemical perspectives to progress into unexplored territory.

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