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Actin-Based Cell Motility and Cell Locomotion

Review

T. J. Mitchison and L. P. Cramer
Department of Cellular and Molecular Pharmacology
University of California, San Francisco
San Francisco, California 94143-0450

Introduction

Most animal cell types possess the capacity to move over or through a substrate, and cell locomotion plays a key role in both normal physiology and disease. Certain cell types are specialized for locomotion, e.g., neutrophils and free living amoebae. In many tissue cells, the capacity for locomotion is normally repressed, but can be activated by wounding or oncogenic transformation. The mechanism of amoeboid (or crawling, or gliding) cell motility has been the subject of scientific scrutiny since the advent of optical microscopy, and many models have been discussed (for the most recent comprehensive review, see Grebecki, 1994). It is now widely accepted that the basic engine for gliding or crawling locomotion is the actin cytoskeleton. An alternative view, that directed lipid flow generates force, has been extensively considered, and rejected, in several recent reviews and we will not discuss it here (e.g., Grebecki, 1994). We will also avoid microtubules, despite their importance for polarity and motility (Vasiliev, 1991). Rapidly moving cells can often move over the substrate without microtubules (keratocytes, neutrophils), but crawling motility always requires actin. The only known exception is nematode sperm cells in which polymers of major sperm protein appear to play the roles of actin filaments (Italiano et al., 1996; Roberts and Stewart, 1995). For the most part, the actin and tubulin cytoskeletal systems function independently; although intercommunication does occur, this would be the subject of an article in itself.

Cell locomotion is undoubtedly complex, requiring coordinated activity of cytoskeletal, membrane, and adhesion systems. Actin filaments themselves are likely to be involved in multiple force-generating mechanisms. To begin a molecular analysis, we need to dissect locomotion into subtypes of motility. A conventional breakdown along spatial/mechanical lines is shown in Figure 1, for a single cell moving over a two-dimensional substrate. Forward motility of the membrane at the front of the cell is called protrusion. It has been intensively studied over the past few years and is probably the aspect of locomotion for which we are closest to uncovering the molecular basis of force generation. Adhesion is required for protrusion to be converted into movement along the substrate. The process leading to forward movement of the nucleus and cell body we have termed traction. This is arguably the most important type of motility for generating overall locomotion, and also the least well understood in either mechanical or molecular terms. The last step in locomotion is comprised of two mechanistically distinct processes: deadhesion and tail retraction. Whether this step is actively motile depends on the cell type. Neuronal growth cones lack tail retraction, instead spinning out an axon as they move, but deadhesion occurs since the axon is more weakly substrate bound than the front of the growth cones (Bray, 1979). Strongly adhesive cells such as cultured fibroblasts tend to have a strongly adherent, extended tail and leave behind a trail of cytoplasmic fragments as they move. Deadhesion/tail retraction may limit movement rate in such cells. In weakly adhesive, fast moving cells such as amoebae and white blood cells, the tail is more rounded and this step is more efficient. Adhesion and its reversal are a major focus of the accompanying review by Lauffenburger and Horwitz (1996 [this issue of *Cell*]), and tail retraction has been discussed elsewhere (Grebecki, 1994). In this review, we will focus on the different structures and potential mechanisms of force generation for protrusion, other leading edge motility, and traction. We will then discuss the relative contribution of each type of motile force to locomotion of the whole cell.

Actin in Protrusive Structures

Protrusive structures at the leading edge of motile cells are highly dynamic and contain dense arrays of actin filaments. In general, where it has been possible to study, these filaments are organized with their barbed ends (fast growing, or plus ends) oriented preferentially in the direction of protrusion (Small, 1988). The simplest protrusive structures are filopodia, thin cylinders that can extend tens of microns from the main cortex (Figure 2). Filopodia contain a tight bundle of long actin filaments oriented in the direction of protrusion. The filaments are held together in the bundle by cross-linking proteins such as fimbrin (Matsudaira, 1994). Filopodia have been most studied in neuronal growth cones, but are present on many other motile cell types. Lamellipodia are thin protrusive sheets that dominate the leading edges of cultured fibroblasts and many other motile cells. The characteristic ruffling appearance of fibroblast leading edges is due to lamellipodia that lift up off the substrate and move backward. The web of actin filaments that shapes lamellipodia is organized as an orthogonal cross-weave between two sets of filaments (Small, 1988) oriented at approximately 45° to the direction of protrusion (Small et al., 1995) (Figure 2). In many cell types, lamellipodia are punctuated at intervals by rib-like microspikes that resemble short filopodia. The orthogonal filaments gather into a tight bundle in microspikes. Amoeboid cells tend to protrude using thicker processes termed pseudopods. The organization of actin in pseudopods has been difficult to study owing to problems with maintaining their organization during fixation. Their cortex is thought to be dominated by a cross-linked mesh of actin filaments with a less polarized organization (Cox et al., 1995) that may be similar to the organization seen in thicker lamellipodia of certain types of growth cones (Lewis and Bridgman, 1992). However, filaments with their barbed ends forward are present in protrusive regions of both these cell types, predominantly on the ventral surface of growth cones (Lewis and Bridgman, 1992), and as far as we can determine from the data presented, in thin tips protruding along the ventral surface of amoeba (Cox et al., 1995).

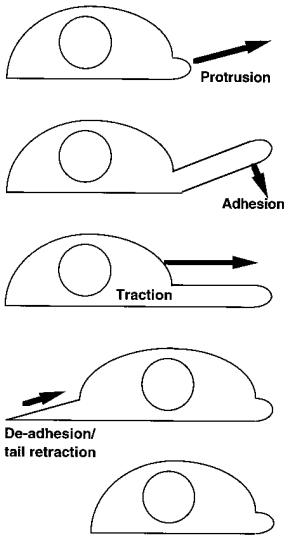

Figure 1. Subtypes of Motility in Locomotion

A single cell moving across a two-dimensional substrate is shown in cartoon form (time increases down the page). Detailed morphology varies between cell types, but the same basic types of motility can be distinguished. In cells where the processes occur simultaneously, the morphology is constant during locomotion.

In all the structures described above, protrusion of the membrane is tightly coupled to polymerization of actin filaments at the leading edge. However, the exact mechanism of actin polymerization and how protrusive force is generated remain controversial. New actin polymer must be generated in one of two ways: by elongation of existing filaments, or by nucleation of new filaments followed by elongation (Figure 3). When pure actin polymerizes, elongation is kinetically favored over nucleation, resulting in long filaments. In motile cells, the situation is complicated by the presence of some actin-binding proteins that affect filament elongation and others that catalyze nucleation of new filaments (Hartwig and Kwiatkowski, 1991; Pollard and Cooper, 1985). Unfortunately, although recent discoveries have been made in motile cells on the signaling molecule control of actin polymerization (Ridley, 1995), it is not yet known whether elongation or nucleation factors are targets for regulation. In the prototypic case of thyone sperm acrosomal process protrusion, Tilney and Inoue (1982) argued that filaments are generated exclusively by elongation of existing barbed ends, originally templated by a stable filament bundle embedded in the sperm nucleus. This results in a bundle of very long actin filaments. In more typical protrusive structures, it has been difficult to measure filament length, but some generalizations can be made. In filopodia, actin filaments are thought to be long, perhaps as long as the filopodium, implying a predominantly elongation mechanism. In lamellipodia of fish keratocytes, Small et al. (1995) have argued that actin filaments may extend the whole length of the lamellipodia, again arguing for a predominance of elongation. However, their methods might have preferentially extracted shorter filaments. Kinetic analysis using fluorescence photoactivation in the same cells suggested that actin filaments turn over very rapidly (Theriot and Mitchison, 1991). This data was interpreted as indicating the presence of a population of short filaments, requiring frequent nucleation. However, this interpretation made assumptions about filament turnover mechanism, and other explanations for the rapid decay in fluorescence signal have been suggested (Small, 1994). The issue is unresolved, but in some cells may be explained by the coexistence of long and short filaments with differential spatial distribution (Lewis and Bridgman, 1992). These controversies highlight the deficiencies in current methods for determining both structure and dynamics of actin filament arrays.

An exciting new direction in research on protrusion

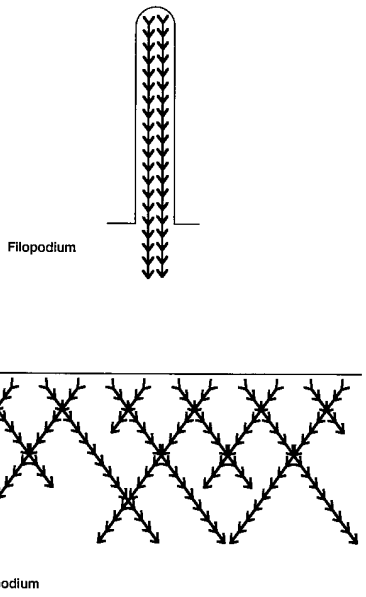

Figure 2. Organization of Actin Filaments in Protrusive Structures

Actin is shown as chevrons and the plasma membrane as a thin line. Filopodia are typically 0.1–0.5 μm thick and extend 5–50 μm. Where measured they contain greater than 15 actin filaments organized as a tight bundle (Lewis and Bridgman 1992). Lamellipodia have a similar thickness and extend 1–15 μm. The extent of organization of filaments in a lamellipodium into a clear cross-weave as shown depends on the cell type (e.g., Cox et al., 1995; Small, 1988).

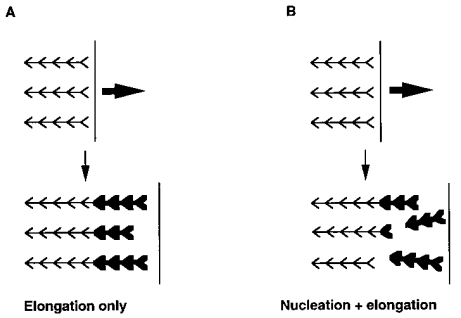

Figure 3. Two Models for Formation of Actin Polymer

New polymer could be generated only by polymerizing onto existing barbed ends (elongation, A) or by a combination of nucleating new filaments and elongating existing ends (B).

has come from studying the intracellular propulsion of certain bacterial and viral pathogens by an actin "comet tail" mechanism that resembles aspects of protrusion at the front of motile cells (Cossart, 1995; Cudmore et al., 1995; Theriot, 1995). The pathogen surface is likely to be biochemically simpler than the inside of the plasma membrane, and thus easier to dissect. It may allow us to distinguish between elongation and nucleation for formation of new actin polymer. One of the bacterial pathogens (Listeria) appears to generate relatively short filaments as it moves (Tilney and Portnoy, 1989; see also Zhukarev et al., 1995), while another bacterium (Rickettsia) generates long filaments (Heinzen et al., 1993). Thus, the balance between elongation and nucleation may vary between these bacteria, and both are compatible with motility. The bacterial motility systems are proving powerful for identifying molecules that regulate the actin cytoskeleton, as has begun to be shown for nucleation (e.g., Cossart 1995).

Regardless of whether new actin filaments are formed by nucleation or elongation, a high concentration of actin monomer is required to drive actin polymerization during protrusion. In the thyone acrosome reaction, a high concentration of stored, unpolymerized actin drives irreversible polymerization. In motile cells, however, new polymerization at the leading edge occurs continually at steady state and must be balanced by depolymerization elsewhere. Actin depolymerization within the protrusive structure is the most likely source of actin monomer, although contribution of actin from other regions in the cell may also play a role. Newly synthesized actin is probably not very important since protein synthesis blocking drugs do not affect protrusion in the short term. Depolymerization of actin filaments may be catalyzed in vivo because filament turnover is faster in vivo than in vitro with pure actin (Theriot and Mitchison, 1991, 1992; Zigmond, 1993). Candidates for this catalysis are proteins in the actophorin/ADF/cofilin family (reviewed by Moon and Drubin, 1995; Sun et al., 1995). How these or similar proteins precisely maintain the monomer pool is not clear. One intriguing possibility is that hydrolysis of actin-bound ATP that accompanies polymerization promotes depolymerization. This would effectively couple depolymerization and maintenance of the monomer pool to the free energy of ATP hydrolysis.

Force for Cell Protrusion

In principle, the force for protrusion could be generated locally at the leading edge, or in the cell body and transmitted to the leading edge by mechanical linkage or hydrostatic pressure. Experiments in amoeba clearly favor local force generation (Grebecki, 1994). Mechanical linkage would require the cytoskeleton to be pushed into the leading edge, but in general, forward movement of actin polymer has not been observed in protrusive structures. In animal cells, there is no evidence for bulk hydrostatic pressure in the cytoplasm, unlike plants where turgor pressure contributes to protrusion. Thus, the evidence strongly favors generation of protrusive force directly at the leading edge.

Proposed mechanisms for generating protrusive force can be divided into those invoking the action of motor proteins to drive protrusion and those in which actin polymerization itself produces force. Force production requires an energy source, and in the cytoplasm, this must ultimately derive from the chemical energy of nucleotide hydrolysis. In motor-based models, a barbed end–directed motor, most likely a myosin, transduces hydrolysis energy directly into force, pushing the membrane tip forward (Figure 4A). Certain myosin I isoforms are enriched in protrusive structures, where they might play the role of the motor (e.g., Baines et al., 1992; Fukui et al., 1989; McGoldrick et al., 1995; Wagner et al., 1992; Wessels et al., 1991), and kinetic analysis of filopodial protrusion has been argued to favor a motor-driven model (Sheetz et al., 1992). Dictyostelium genetics is beginning to reveal specific roles for individual myosin I isoforms. So far, neither single nor multiple myosin mutants has blocked protrusion in these cells, though it is too early to rule out any role for myosins (e.g., Jung and Hammer, 1990; Titus et al., 1993; Wessels et al., 1991). Protrusion of hyphae is blocked in Aspergillus nidulans null for myoA, a myosin I–like protein, but it is not clear if this is due to a direct effect on the actin cytoskeleton or due to reduced vesicle transport to the growing tip (McGoldrick et al., 1995).

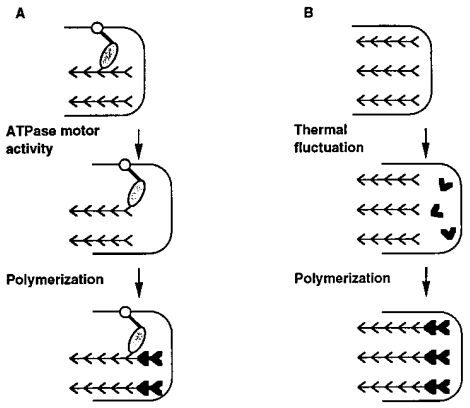

Figure 4. Two Models for Generation of Protrusive Force

In the ATPase motor model (A), forward movement of the membrane tip is driven by a motor protein (e.g., myosin I) moving toward filament barbed ends. Polymerization then fills in the resulting gap. In the thermal ratchet model (B), gaps between the protruding membrane and the barbed ends of actin filaments are created by thermally driven movements (Peskin et al., 1993). These could be either fluctuations in the position of the membrane, or in the length of the actin filaments (Peskin et al., 1993). Polymerization then fills in the gap, preventing backward movement of the membrane.

The idea that polymerization alone could push a membrane forward is consistent with the polarity of actin filaments in protrusive structures, but perhaps less conceptually obvious than using a motor protein. However, there is considerable evidence in favor of such a model. Polymerization of pure actin inside a lipid vesicle can deform the membrane (Cortese et al., 1989) and polymerization of other proteins including tubulin (Hotani and Miyamoto, 1990) can also produce membrane-deforming force. Any proposed mechanism for coupling polymerization to protrusion can take advantage of actin-binding proteins that cross-link and bundle the newly formed polymer (Matsudaira, 1994) increasing the stiffness of the gel (Condeelis, 1992). Polymerization may

be coupled to ATP hydrolysis by maintenance of the monomer pool, as described above, but how is polymerization in turn coupled to physical protrusion of the membrane? Two mechanisms have been proposed for physical coupling, the simpler of which is the thermal ratchet model (Peskin et al., 1993; Figure 4B). In this model, the energy to drive protrusion comes from polymerization, but the rate is limited by the probability that thermal fluctuation will allow a subunit to be added. A serious objection to the model concerns the mechanical properties of the plasma membrane, which may be too stiff to deform at the required rate by thermal fluctuations alone. However, another potential source of fluctuation is thermally driven changes in the effective length of the actin filaments due to temporary bending (Peskin et al., 1993). The alternative potential mechanism of mechanical coupling of polymerization to protrusion is through local osmotic effects due to gel swelling. Polymerizing actin may release water and bound ions, promoting water influx into the polymer gel (Condeelis, 1992).

Currently, we lack decisive evidence to decide between motor protein-driven and polymerization-driven models for protrusive force generation, and it may turn out that both are important. The same distinction has been difficult to make in the microtubule-dependent process of anaphase chromosome movement (Inoue and Salmon, 1995). One problem with analyzing the role of myosins in protrusion has been the coexistence of many members of this protein family in cells, complicating genetic analysis, and the lack of pharmacological inhibitors. An interesting pharmacological tool is the drug butane-dione-monoxime (BDM), a low affinity inhibitor of myosin ATPase that readily enters cells. BDM inhibits myosin II and myosin V in vitro and may inhibit all myosins (Cramer and Mitchison, 1995). Listeria, as a model for lamellipodia protrusion, does not stain for known myosins, and its motility is neither inhibited by BDM nor appears to utilize osmotic forces. Although other ATPase actin motors could exist, the data makes us prefer the thermal ratchet mechanism for Listeria. Two evolutionary arguments also support thermal ratchets. First, in nematode sperm motility, actin is replaced by another polymerizing protein, MSP. This is surprising enough, and it is difficult to imagine that novel motors have also evolved in this system. Second, using ATP hydrolysis by actin to power thermal ratchet based protrusion provides the simplest possible mechanism, and the easiest one to imagine evolving in primitive eukaryotes (Mitchison, 1995). Proponents of polymerization-driven protrusion need to provide alternative roles for the myosin I isoforms that are enriched in leading edges. Video tracking has shown that certain membrane proteins can move rapidly forward in lamellipodia (Kucik et al., 1989; Sheetz et al., 1990; Schmidt et al., 1994). This is faster than that allowed by diffusion and could be driven by myosin I, allowing receptors to rapidly promote substrate sensing and adhesion (Figure 5C). Alternatively myosin I may have some role in stabilization of protrusive structures as implicated for brush border myosin I (Temm-Grove et al., 1992) or in vesicle transport/secretion (McGoldrick et al., 1995).

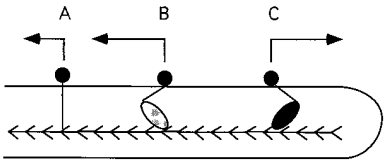

Figure 5. Particle Motility in Protrusive Structures

Surface attached particles and receptors (and intracellular particles; data not shown) move inward with respect to the substrate by passive attachment to inwardly moving actin (A), or are actively transported by a putative minus end–directed actin-based motor (B). A myosin I may transport particles and receptors that have been observed to move outward (Kucik et al., 1989, Sheetz et al., 1990) (C). In some cells, actin does not move with respect to the substrate in lamellipodia (Theriot and Mitchison, 1991). In these cells, particles may move on stationary actin by (B) or (C). For simplicity, we have not indicated how the actin may be moving or why it does not move forward in (B).

Backward and Sideways Motility in Protrusive Structures

Understanding forward motility of lamellipodia and filopodia is complicated by the continuous backward movement of internal structures and surface-attached particles. This type of motility as viewed by high resolution, time lapse microscopy is one of the most dramatic properties of lamellipodia and filopodia and is seen in all thin leading edge structures of motile cells. Backward movement in lamellipodia of motile cells has been studied for decades and often proposed to play some role in locomotion, although backward movement is not immediately reconcilable with forward movement of the cell. Are we closer to understanding what is moving backward and how the movement is powered? Now classic photobleaching studies of Wang (1985) and cytochalasin addition experiments of Forscher and Smith (1988) lead most authors to conclude that all the actin filaments in lamellipodia and filopodia are moving continually backward toward the cell body, and that backward movement of particles and surface receptors is driven by coupling to this flow of moving actin (Figure 5A) (Mitchison and Kirschner, 1988; Sheetz et al., 1989; Smith, 1988). Much of the evidence still favors such a model, though direct tests have yielded ambiguous results. Filaments marked by photobleaching of phalloidin in growth cones move at the same rate as surface-attached beads (Lin and Forscher, 1995), but actin filaments in tissue culture lamellipodia marked by fluorescence photoactivation move backward at only one third the rate of particles and surface-attached beads (Theriot and Mitchison, 1992). Different rates of backward movement might simply reflect the presence of different populations of actin filaments that are not equally detected by current methods of observation, but are moving backward at different rates in the same lamellipodia. Consistent with this possibility is the observation that particles move backward at different rates over dorsal and ventral surfaces respectively in primary fibroblasts (Harris and Dunn, 1972). Alternatively, these data also fit a model in which some particles or surface-attached beads are passively attached to moving actin and others are actively driven backward at a faster rate in the same lamellipodium, perhaps by the action of a

pointed end–directed actin-based motor (Figures 5A and 5B), a possibility we take seriously. Similar, but generally slower, backward movement of particles occurs over the cell body (capping), and this is known to be driven by myosin II (Pasternak et al., 1989). However, myosin II is absent from lamellipodia and in Dictyostelium does not by some mechanism (e.g., raking backward) drive backward movement of particles on protrusive structures (Jay and Elson, 1992). Roles for myosin I's have not been reported in the literature. The prevalent model in which all backward movement in leading edge structures reflects coupling to moving actin filaments needs to be tested more rigorously, and this may require development of new probes of actin dynamics and particle movement.

Sideways movements of microspikes and filopodia in lamellipodia have received less attention than backward movements, though they can be dramatic and must provide clues to dynamic organization of actin filaments. Sideways movement may be important in growth cone guidance, allowing the same filopodium to move around a growth cone as it samples the environment (Bray and Chapman, 1985). Small (1994) has pointed out a geometric relationship between orientation of actin filaments in protruding lamellipodia and sideways movements of their elongating barbed ends. This idea fits with the graded radial extension models of keratocyte movement (Lee et al., 1993), but it cannot explain rapid sideways movements of microspikes in lamellipodia that are protruding slowly with respect to sideways movements (Fisher et al., 1988). The mechanism of this movement is enigmatic: we might speculate a role for motors moving along actin filaments that are arranged at an angle to the direction of protrusion and thus generate a force component normal to the protrusive direction.

Actin in the Cell Body

Once we move back from the leading edge to the cell body, actin organization is less well understood. We define the cell body as the thickest region of cytoplasm containing the nucleus together with a region of intermediate thickness (often called the lamella) found between the nucleus and protrusive structures. As with protrusive structures, organization and polarity of actin filaments in the cell body has important implications for mechanisms of force generation. We know from electron microscopy of lamellae of motile animal cells that cortical actin (subplasmalemma) appears as a loose meshwork of apparently short, randomly organized filaments. Fluorescent studies show that cell bodies of motile animal cells also have bundles of actin filaments, but unlike the bundles in lamellipodia, their organization and polarity are generally not known. It is known that bundles of actin filaments are commonly homopolar in plant cells where they provide the substrate for myosin-driven cytoplasmic streaming (Kuroda, 1990). Also neuronal growth cones have long homopolar actin filament bundles with barbed ends primarily in the direction of protrusion, but it is not clear whether this organization extends into the cell body of the growth cone (Lewis and Bridgman, 1992). Actin filament bundles in cell bodies of motile animal cells are almost always loosely termed "stress fibers" in the literature.

Stress fibers have a strict definition in that they are organized like muscle sarcomeres, with short actin filaments arranged in bundles of alternating polarity interspersed with bipolar myosin II filaments. Most fluorescent studies of motile cells do not determine whether observed bundles of actin filaments are strictly stress fibers by S1 decoration and the possibility exists that some of these actin fibers have a different organization. Certain locomoting cells that lack stress fibers have thinner actin bundles often oriented in the direction of locomotion (Couchman and Rees, 1979). The polarity of these filaments are not known, but the α-actinin fluorescent staining on similar filaments in related cells does not appear characteristic of sarcomeres (Tomasek et al., 1982). These thin actin bundles in animal cells have received little attention, despite their possible importance for locomotion.

Generation of Traction Force

Many models have been postulated for generation of traction force, but we will concentrate on two possibilities that propose roles for myosin II in force generation. One reason for focusing on this molecule is its abundance in the cell body of motile cells (Huxley, 1973); another is its proven importance in locomotion. Much attention was paid to the observation that genetic ablation of myosin II in Dictyostelium did not completely block cell locomotion or chemotaxis (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). Since myosin II is not involved in protrusion, a block to this type of motility would not be expected. Less celebrated is the observation that the net rate of locomotion (reflecting traction) was greatly reduced in myosin II nulls, and in the physiological setting of the slug stage, the nulls are completely blocked in locomotion (Doolittle et al., 1995). That this is direct evidence for myosin II generating traction force is supported by the reduced locomotion of the nulls on more adhesive substrates (Jay et al., 1995).

How does myosin II generate traction force? Two fundamentally distinct mechanisms are conceivable, based either on contraction or transport. The difference concerns the source of polarity that makes the cell move forward. In contractile models (Figure 6A), force is generated as tension, which has equal components pulling the cell forward and backward. Net locomotion occurs by superimposing either polarized adhesion, stronger at the front relative to the back, and/or polarized assembly/disassembly of the cortex. A related contractile model is based on polarized cortical contraction/relaxation (Bray and White, 1988). Transport models postulate that myosin generates force in an inherently polarized manner, acting directly to pull the cell forward (Figure 6B). Transport models, in contrast with contraction models, require a net polarity in actin filaments that are attached to the substrate, which act as tracks over which the rest of the cell is pulled. Polarized adhesion and assembly may also be needed for locomotion, but they are no longer the primary source of polarity.

Contraction-driven motility was one of the earliest types of force proposed for amoeba locomotion and has been debated since early last century (see Bray and

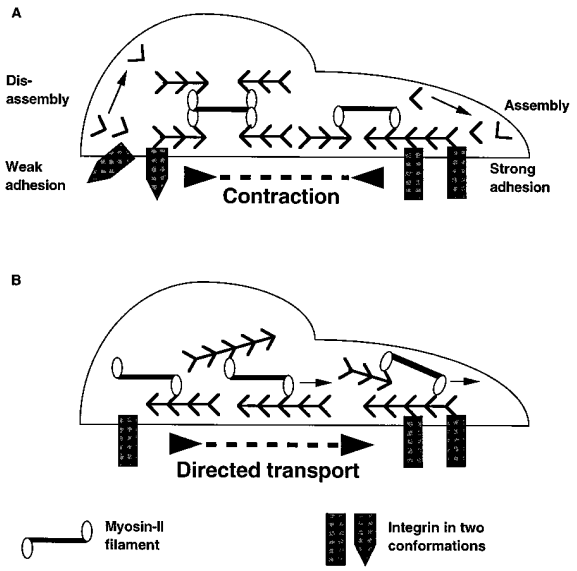

Figure 6. Two Models for Generation of Traction Force Using Myosin II Activity

In the contraction model (A), myosin pulling on filaments of opposite polarity creates a cortical tension that pulls the cell equally in all directions. This contraction can be converted into movement by combining it with preferential assembly of the cortex at the front of the cell and disassembly at the back, and/or by regulating the relative strength of adhesive contacts to the substratum at the front and back. In the transport model (B), myosin activity pulls the body of the cell over an oriented track of actin filaments attached to the substratum.

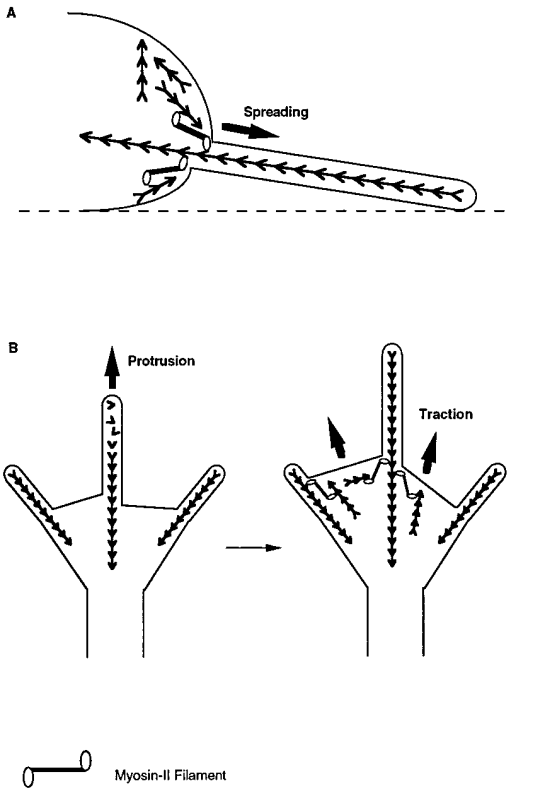

Figure 7. Transport-Driven Traction in Two Types of Cell Motility

(A) shows a side view of a PtK2 cell respreading after mitosis. The oriented track of filamentous actin is provided by retraction fibers. Experimental evidence support a model in which myosin II drives spreading by pulling the cell over these retraction fiber filaments (Cramer and Mitchison, 1993, 1995).

(B) shows an extension of this model to growth cone locomotion. Protrusion of filopodia is driven by the polymerization mechanisms discussed above, and is followed by adhesive contact of the filopodium to the substratum. The growth cone is then shown as being pulled outward over this oriented filamentous actin track provided by myosin II activity. There is no direct experimental support for such a model in growth cones, though myosin II is present at the base of filopodia (Bridgman and Dailey 1989) and homopolar bundles of actin filaments have been observed in growth cones (Lewis and Bridgman, 1992).

White, 1988; Grebecki, 1994; Sheetz, 1994). Credence for the idea came much later when it was determined that actin and myosin were present in nonmuscle motile cells, and a model for movement based on known striated muscle contraction was suggested (Huxley, 1973). Myosin II driven contraction is thought to be responsible for generating cortical tension, an important factor in shaping cells. Myosin II is also responsible for local contractions, e.g., cytokinesis. Although less is known about a role for contraction in driving locomotion there are reasons to favor it. Preferential assembly of the cortex probably does occur at the front of the cell, where the leading edge meets the cell body and where polymerization of myosin II filaments is thought to occur (Kolega and Taylor, 1993; McKenna et al., 1989). Polarized adhesion, stronger at the front of the cell than the back, is supported by direct measurements in some cells, as discussed by Lauffenburger and Horwitz (1996). One situation where there is strong evidence for contraction-based forward movement is "purse string" motility (discussed in Cramer et al., 1994).

Transport mechanisms have been discussed less in the literature, presumably because of the apparent absence of information on the polarity of actin filaments in the cell body of locomoting cells. When homopolar actin bundles are present in motile cells and oriented in the direction of cell movement, it is natural to propose that they could act as transport tracks. One such situation occurs when certain tissue culture cells respread after mitosis (Figure 7A). The spreading cell moves outward preferentially along retraction fibers that provide a homopolar transport track (Cramer and Mitchison, 1993, 1995). Inhibition of spreading by the myosin inhibitor BDM suggests that spreading may be driven by myosin activity, and this is supported by immunolocalization of myosin II at the spreading edge (Cramer and Mitchison, 1995). Another situation where transport tracks may exist is where filopodial actin bundles extend into growth cones. Indeed, long actin filament bundles with barbed ends mostly in the direction of forward movement are located on the ventral surface of growth cones (Lewis and Bridgman, 1992). Pulling force produced by filopodia (the "contractile force" of Heidemann et al., 1991) may be generated by myosin pulling the growth cone body forward on these bundles. Myosin II is present in the correct location to generate such force (Bridgman and Dailey 1989). In this view, growth cones (and other

filopodia-rich cells such as sea urchin secondary mesenchyme cells) could first use protrusive force to send out filopodia that then act as transport tracks for myosin to pull the growth cone forward (Figure 7B). This model may be more generally applicable. For example, the uniform actin deposited by fibroblast lamellipodia may act as a transport mat for myosin to pull the cell body forward. While the generality of transport based mechanisms for generating traction force is not clear, it does seem likely that they are at least as important as contraction based mechanisms for some types of cell motility.

What experiments might distinguish contraction from transport-based traction force in locomoting cells? The contraction model predicts that actin filaments can slide or shear over relatively short distances, but are essentially fixed with respect to the substrate over the longer distances a locomoting cell is expected to move. This is consistent with morphological observations suggesting that the cortex is fixed with respect to the substrate in moving embryonic cells (Bilozur and Hay, 1989). Actin filament marking experiments could test the fixed cortex and related contraction-based models in locomoting cells. Plausibility of the transport-based model hinges on detection of actin filaments preferentially oriented with barbed ends in the direction of forward movement under cell bodies of locomoting cells.

Integration of Motile Forces for Cell Locomotion

We have discussed three types of force-generating systems, and the question naturally arises as to their relative importance for locomotion as a whole. For example, is protrusive force in lamellipodia ever enough to power cell locomotion alone? Is force derived from backward movements in protrusive structures important for forward locomotion, and if so in which cells? Can cells locomote purely by traction force? We suspect the relative importance of these forces depends on such factors as the rate of locomotion, distance the cell has to travel and strength of cell adhesion.

Fast moving cells such as keratocytes and leucocytes can locomote 10–60 times faster than a typical primary fibroblast and are 3–20 times less adhesive (Oliver et al., 1994). Thus, the cell bodies of keratocytes and leucocytes impose little drag force during cell protrusion. In these cells, force from cell protrusion may be sufficient to move the entire cell forward, provided the lamellipodia makes adhesive contact with the substrate. Protrusion at the leading edge would generate membrane tension that could pull the back of the cell forward. Estimated protrusive force from rates of actin polymerization can account for measured locomotory force in these cells (Oliver et al., 1994). However, a contractile force normal to the direction of locomotion has also been observed in keratocytes (Lee et al., 1994), and this could also contribute to forward locomotion. Nematode sperm, another fast moving, weakly adhesive cell type, may also move by protrusion alone, in this case driven by MSP polymerization. No other potential sources of motile force are known in these cells (Roberts and Stewart, 1995).

The clearest example where backward motility in lamellipodia appears to couple directly to forward locomotion comes from work on Aplysia growth cones by Lin and Forscher (1995). As the rate of growth cone locomotion increases, the rate of backward movement (measured relative to the substrate) decreases. This is consistent with a model in which backward movement of actin filaments is attenuated by coupling to the substrate, generating instead force to pull the growth cone forward. In contrast, backward movement of actin filaments in protruding lamellipodia of stationary fibroblasts is not directly related to protrusion (Theriot and Mitchison, 1992). This difference might arise because fibroblast lamellipodia are often detached from the substrate, preventing efficient coupling of actin filaments to the substrate.

For groups of cells traveling relatively short distances or undergoing morphogenetic movements during development, contraction-based motility can occur in the absence of lamellipodia protrusion and is probably sufficient for active movement, e.g., purse string contraction (discussed in Cramer et al., 1994). For individual cells traveling greater distances, such as fibroblasts and neurons, lamellipodia and/or filopodia are always observed to protrude as the cells locomote and the question arises as to the relative importance of protrusion and traction. We picture that traction is an inherently stronger force and is therefore likely important for cells that are more strongly adherent to the substrate, which thus locomote more slowly. For these cells, we do not know whether protrusive force contributes to the overall force for locomotion, or whether protrusion is mainly required to lay down a polarized actin track for myosin as proposed in Figure 7B.

Future Directions

For each of the forces, we have discussed multiple molecular mechanisms, and one obvious priority for future work is to distinguish between them. To do this, we will need better ways of analyzing the structure and dynamics of the actin filament arrays in cells. In particular, better filament marking experiments in live cells and filament polarity measurements in fixed cells are needed to test traction models. Analyzing the role of specific motors is another important challenge for the future. To do this, we will need, in addition to genetic approaches, better pharmacological tools if we hope to study motors in a variety of cell types. It may turn out that some of the mechanisms set up as alternatives in this review in fact turn out to be both true, to an extent that varies with cell type. Applying what we have learned from cells locomoting on a two-dimensional substrate to those moving through a three-dimensional matrix is another important direction. The whole issue of regulation that we have barely touched on will have to be integrated with force-generating mechanisms to understand how locomotion is directed. Overall, we foresee the basic mechanisms of cell locomotion being solved, perhaps in this century. Using this information to manipulate locomotion for therapeutic benefit appears to be a more daunting task. It is, however, one well worth pursuing given the importance of locomotion in many aspects of physiology.

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