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In pursuit of myosin function

Minireview

James A. Spudich
Departments of Cell Biology and
Developmental Biology
Stanford University School of Medicine
Stanford, California 94305

Introduction

Myosin, first identified in muscle extracts by Kuehne more than a century ago (Kuehne, 1864), is an actin-based molecular motor that has proven to be a key component of several fundamental forms of movement in nonmuscle cells. This molecule is involved in nearly every aspect of cell regulation, from events involved in cell-cell signaling and chemotaxis to the highly regulated changes in cell shape that occur during cytokinesis. Myosin consists of two heads, each called Subfragment 1 or S-1, connected by an α-helical coiled-coil tail, which is involved in the formation of thick filaments. Highly purified S-1 moves actin filaments in vitro (Toyoshima *et al.,* 1987; Manstein *et al.,* 1989a), and it produces a force in vitro (Kishino and Yanagida, 1988) which is near that expected from force-velocity measurements with intact muscle. Thus S-1 contains all of the domains needed for motor function.

Multiple approaches have been used to implicate myosin in a variety of nonmuscle movements such as cytokinesis, karyokinesis, cell migration, capping of surface receptors, and morphogenetic shape changes, associated with development (for reviews, see Warrick and Spudich, 1987; Korn and Hammer, 1988). Recent molecular genetic experiments have aided significantly in the elucidation of the roles of myosin in vivo, and the eukaryote *Dictyostelium discoideum* has proven to be a good meeting ground for cell biologists, physiologists, biochemists, and geneticists interested in the molecular mechanisms of cell and developmental biological events. This organism, although nearly indistinguishable from a leukocyte or other higher eukaryotic cell in many aspects of its behavior, is haploid and has a relatively simple genome. Its virtues for biochemical, molecular genetic, cell biological, and developmental studies have been described in detail (Loomis, 1982; Spudich, 1987; Devreotes and Zigmond, 1988). The discovery of highly efficient gene targeting in *Dictyostelium* (De Lozanne and Spudich, 1987) and the use of the antisense RNA approach in this organism (Knecht and Loomis, 1987) have provided the opportunity to explore the in vivo roles of myosin in cell and developmental biology. The phenotype of mutant *Dictyostelium* cells that are missing myosin is summarized here. Surprisingly, some of the cell behaviors thought earlier to require myosin, such as cell migration, do not, while others, such as cytokinesis, clearly do. Other cellular behaviors, while not absolutely requiring myosin, are modified by it in important ways.

Although the conventional myosin is the best studied of all molecular motors, new force-transducing molecules, both actin-based and microtubule-based, have been discovered in the last decade. Thus the advent of in vitro assays for molecular motion resulted in the discovery of kinesin (Vale *et al.,* 1985) and cytoplasmic dyneins (Vallee *et al.,* 1988), both of which are microtubule-based and move in opposite directions along microtubules, and revealed that the unusual myosin-like molecule from *Acanthamoeba* (Pollard and Korn, 1973), called myosin I, is capable of moving along actin filaments (Albanesi *et al.,* 1985). Generally, the term myosin has continued to be used in the literature to refer to the conventional myosin and will be used throughout this minireview to refer to this form of actin-based motor. Other forms are given specialized names, such as myosin I, to distinguish them from the myosin first discovered and named long ago by Kuehne.

This is a minireview and, as such, is not comprehensive. I will not address here the microtubule motors, which are thought to play important roles in cell behavior (Warner *et al.,* 1989), nor will I summarize all that is known about actin-based motors. Rather, I promote the molecular genetic approach and applied to a particular organism, *Dictyostelium discoideum,* which is being used to better understand the nature of the diverse actin-based motors and their roles in cell and developmental biology. Clearly, not every conclusion regarding how *Dictyostelium* carries out various cell functions can be applied to every other cell,

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and it is important that many different cell types be studied. The essential features of how cells carry out their cellular and developmental functions, however, are not likely to be very different from one eukaryote to another. A complete understanding of cell regulation will require both the continued use of a healthy diversity of organisms as well as a considerable increase in focus on a select few.

Molecular genetic approaches in *Dictyostelium* have provided for dissection of the roles of myosin in vivo

Determination of the structure and function of cellular proteins is the primary focus of cell and developmental biology. How does one sort out the in vivo functions of these proteins? To be sure, multiple approaches are required, but the lessons of the last thirty years of prokaryotic biology teach us that one essential approach is the use of molecular genetics and classical genetics. The further development of these techniques in eukaryotic cell biology and developmental biology will not only transform the kinetics of advancements in these areas, but will provide definitive answers to questions of protein structure/function relationships heretofore not available. My laboratory and others have taken on the challenge of using molecular genetics to elucidate the roles of conventional myosin and other potential actin-based motors in nonmuscle cells. Although yeast is clearly one of the best genetic systems for elucidation of the function of eukaryotic proteins (for reviews, see Drubin, 1989; Katz and Solomon, 1989), I focus here on *Dictyostelium discoideum,* a cell that, unlike yeast, exhibits cell movements and changes in cell shape like those apparent in higher cells but still is readily manipulated by molecular genetic and even classical genetic techniques.

Myosin is required for several fundamental forms of cell movement

In the mid-1980s, the coding portion of the *Dictyostelium* conventional myosin heavy chain gene was cloned and shown to be a single-copy gene (De Lozanne *et al.,* 1985, 1988), and its entire sequence was obtained by Warrick *et al.* (1986). This advance provided the basis for two approaches to create a *Dictyostelium* cell that specifically lacks myosin. The isolated gene was used by Knecht and Loomis (1987) to create antisense RNA that reduced the level of expression of myosin in the cell to <1% of wild type levels. In complementary experiments, the gene was used by De Lozanne and Spudich (1987) to carry out disruption of the myosin gene by homologous recombination. This disruption gave rise to a cell with <0.1% of wild type levels of intact myosin, which was replaced with the expression of a head fragment of the myosin molecule. More recently, Manstein *et al.* (1989b) deleted the myosin gene by homologous recombination with the use of linearized vectors, which created cells that are totally depleted of conventional myosin. Analysis of these various cell lines has given us considerable insight into the in vivo role of myosin.

Cytokinesis requires myosin. In all of the *Dictyostelium* molecular genetic experiments described above, the myosin-minus cells failed to divide in suspension and became large and multinucleated, indicating a defect in cell division. Analysis of cells by computer-enhanced video microscopy show that the myosin-minus mutants fail even to initiate the furrow associated with cytokinesis (J.A. Spudich and S.J. Kron, unpublished observations). The results from the *Dictyostelium* molecular genetic experiments provided the first genetic proof that myosin is required for cytokinesis, and they confirmed the view, derived from immunofluorescence, electron microscopy, and anti-myosin injection experiments (Schroeder, 1973; Mabuchi and Okuno, 1977; Fujiwara and Pollard, 1978; Kichart *et al.,* 1982), that a contractile ring of actin and myosin is involved in constricting the cell into two daughter cells during mitosis. Interestingly, if the myosin-minus cells are grown on a surface, they are able to attach to and migrate on that surface (see below) and undergo what I have termed traction-mediated cytoplasmic fission, or cytofission for short. The external traction forces operating on the cells cause them to fragment into smaller pieces (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987).

One of the virtues of the gene replacement approach is that one can examine a cell that has had its myosin altered in some interesting way and then ask whether and how cytokinesis is altered. De Lozanne and Spudich (1987) created *hmm* cells, in which the intact myosin was replaced with a myosin fragment that is missing the portion of the tail responsible for thick filament formation. Such a truncated myosin has been purified and shown to retain functional ATPase-containing heads (Ruppel *et al.,* 1989). Is the motor function sufficient for cytokinesis to occur? These *hmm* cells fail to undergo cytokinesis, indicating that the tail portion of the myosin that is involved in thick filament formation is essential for the fundamental process by which two daughter cells normally form.

Capping of surface receptors and concomitant cortical tension changes are generated by myosin. The role of myosin-dependent contractile forces in the active movements of cell membrane proteins has been the subject of much controversy (Bretscher, 1984; Singer and Kupfer, 1986; Bray and White, 1988; Bretscher, 1988; Forscher and Smith, 1988; Sheetz *et al.,* 1989). A direct test of myosin involvement in these processes was provided by the availability of myosin-minus mutants. The response of wild type *Dictyostelium* cells to the tetravalent lectin Concanavalin A (Con A) is similar to that of lymphocytes (Condeelis, 1979; Pasternak *et al.,* 1989b), again highlighting the similarity of this "simpler" cell to higher eukaryotes. In both cell types, the Con A-bound receptors patch on the cell surface and then form a cap at one end of the cell in a process known to require ATP. Concomitant with the formation of the cap is a transient increase in cortical tension, measured as an increase in cell stiffness (Pasternak and Elson, 1985). Comparison of *Dictyostelium* wild type cells to the myosin-minus mutants established that myosin is essential both for capping of cell surface receptors crosslinked by Con A and for the concomitant increase in cell stiffness seen in wild type *Dictyostelium* (Pasternak *et al.,* 1989b). Patching of crosslinked receptors is still apparent in the myosin-minus mutants, but the patches fail to cap. Furthermore, depletion of cellular ATP by azide causes a contraction in wild type cells that causes them to stiffen and become spherical, possibly because of a "rigor" contraction of a cortical shell of actin and myosin. These responses are absent in the myosin-minus mutants. It is clear, therefore, that contractile tension generated by myosin can drive both a change of cell shape and the capping of crosslinked surface receptors. The *hmm* cells also fail to cap (Y. Fukui, A. De Lozanne, and J.A. Spudich, unpublished observations), showing that, as for cytokinesis, the tail portion of the myosin molecule is required for this process to occur.

Myosin is required for morphogenetic changes of multicellular assemblies during *Dictyostelium* development. *Dictyostelium* has a well-characterized developmental cycle (Loomis, 1982; Spudich, 1987). Starvation initiates a developmental program that involves the aggregation of many cells by chemotaxis toward an external cyclic AMP (cAMP) signal. The program culminates in the formation of a fruiting body consisting of two cell types, stalk cells and spores. In the myosin-minus mutants, this developmental program is blocked in an orderly and complete fashion at a rather precise stage. Surprisingly, the mutant cells can migrate on a surface and even respond to cAMP by chemotaxis, although they do so at much-reduced efficiency (see below). These motile behaviors lead to the formation of multicellular mounds, but these aggregates fail to differentiate further. Thus the morphogenetic shape changes associated with the latter part of the developmental cycle show an absolute requirement for myosin. Interestingly, addition of wild type cells to the population of myosin-minus cells rescues the mutant cells and carries them through the remainder of development, resulting in haploid spores that are genotypically myosin-minus (De Lozanne, 1988; Knecht and Loomis, 1988).

Myosin is not required for other important forms of cell movement

Cell-surface extensions and cell migration. From various studies of the last twenty years, especially cellular localization experiments using indirect immunofluorescence, myosin was postulated to be a positive force transducer for cell migration. For example, observations that myosin is predominantly located in the posterior end of migrating cells of various types, including *Dictyostelium* (Yumura and Fukui, 1985), fit with the concept that internal pressure resulting from a myosin-dependent contraction in the rear of the cell may force the cell outward in the form of extensions in the front (e.g., Mast, 1926; Taylor and Fechheimer, 1982).

It was somewhat surprising, therefore, to discover that *Dictyostelium* cells lacking myosin are perfectly capable of extending filopodial and pseudopodial projections resulting in cell-surface ruffles and cell migration on a surface. Indeed, the mutant cells appear to extend pseudopodia even more prolifically than do wild type cells, although the rate of expansion and final area of the pseudopodia are somewhat lower than those of wild type cells (Wessels *et al.,* 1988).

Intracellular particle movements. Movements of intracellular particles are readily apparent in the *Dictyostelium* myosin-minus mutants. Careful examination to distinguish between various types of particles, such as small vesicles, mitochondria, and other organelles, has not been carried out. A recent study by Wessels and Soll (personal communication) using a computer-assisted dynamic morphology system shows that some particles move more slowly by about a factor of two in the myosin-minus cells. This change in rate suggests that myosin can influence the velocity of intracellular particle movements, but one can-

not conclude that it is doing so by attaching to the particles and pulling them along actin filaments within the cell. The lack of myosin may indirectly affect the integrity of the cytoplasmic cytoskeleton and therefore change the degree of sieving of particles as they move through the cytoplasmic matrix.

Karyokinesis. One of several theories of the last two decades of how chromosomes move toward their poles during the anaphase stage of mitosis involved myosin as the motor. Myosin has been localized in spindles by immunofluorescence (e.g., Fujiwara and Pollard, 1978). One hypothesis has been that the microtubules in the spindle may serve the role of a governor, disassembling at a slow rate and thus controlling the speed of the myosin engine pulling on the chromosomes (Nicklas, 1988). The most striking evidence in support of this theory was the observation by Cande *et al.* (1977) that anti-actin not only stains the spindle of mammalian cells but that the staining appears to be restricted to a specific region of the spindle, the kinetochore fibrils, throughout anaphase. This restricted localization to that part of the spindle that is specifically associated with the chromosomes during movement to the poles seemed to be good support for the model, but numerous other studies have supported models that rely solely on the microtubule system, with or without microtubule-based motors (Mitchison, 1988; Nicklas, 1988).

Anti-myosin injection experiments in fertilized echinoderm eggs cast the most serious doubt on myosin-driven chromosome movement. The antibody inhibited cytokinesis without inhibiting karyokinesis (Mabuchi and Okuno, 1977; Kiehart *et al.,* 1982). These experiments, while providing strong support for the argument that myosin is not required for karyokinesis, could be misleading if the putative myosin in the spindle were not sufficiently accessible to the antibody. Unlike myosin in the contractile ring, myosin could be sequestered in a relatively inaccessible form in the dense array of microtubules and membranous elements of the spindle. Furthermore, only a few molecules of myosin would be needed on energetic grounds to pull chromosomes to the poles (Sheetz and Spudich, 1983; Nicklas, 1988), so the antibodies would have to be extremely thorough in their inactivation of the motor molecules. Negative results with antibody injection experiments, therefore, cannot be conclusive. Thus it was critical to examine this question using the molecular genetic approach, which offers the ultimate proof whether the phenotype of the relevant mutant is definitive for or against a considered model. The molecular genetic experiments with *Dictyostelium* showed that myosin is, indeed, not required for karyokinesis. The cells appear to divide their nuclei at nearly normal rates, and the cells become large and multinucleated (Knecht and Loomis, 1987; De Lozanne and Spudich, 1987; Manstein *et al.,* 1989b). This is a situation where a negative result can be as important and illuminating as a positive one, for the genetic experiments with *Dictyostelium* constituted genetic proof that myosin, while required for cytokinesis, is not required for karyokinesis.

Myosin modulates chemotaxis, possibly by affecting cell polarity

That cell polarity is considerably reduced in myosin-minus cells has been noted by numerous workers (Wessels *et al.,* 1988; Y. Fukui, unpublished observations). The myosin-minus cells tend to extend pseudopodial extensions over most of their surface. As illustrated in Figure 1, this loss of polarity might explain the significant reduction in the efficiency with which the myosin-minus cells chemotaxe toward cAMP (Wessels *et al.,* 1988). Wessels *et al.* (1988) examined wild-type and mutant *Dictyostelium* cells with the use of a computer-assisted dynamic morphology system to measure a chemotactic index, which is calculated from a centroid track of cells as the net distance moved toward the source of chemoattractant divided by the total distance traveled by the cell to get to that point. They found the chemotactic index of mutant cells to be only one-fifth that of wild-type cells. So myosin clearly serves an important modulating function in this process.

It should be noted that myosin is not absolutely required for cell polarization or for chemotaxis in *Dictyostelium* (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Manstein *et al.,* 1989b). The complex signal-transduction responses that the chemoattractant (cAMP) elicits in wild-type cells, such as down-regulation of the cAMP receptor, activation of adenylate and guanylate cyclase, and secretion of cAMP, all occur in myosin-minus cells (Peters *et al.,* 1988). Furthermore, the mutant cells retain the ability to move up a gradient of cAMP, although they do so with much-reduced efficiency. Our working hypothesis is that myosin provides the cell with the appropriate degree of polarity to allow it to move efficiently in a directed way to the source of chemoattractant.

A model for polarity generation in a chemotaxing cell. A highly polarized wild type *Dictyostelium* cell, such as that undergoing chemotaxis, has

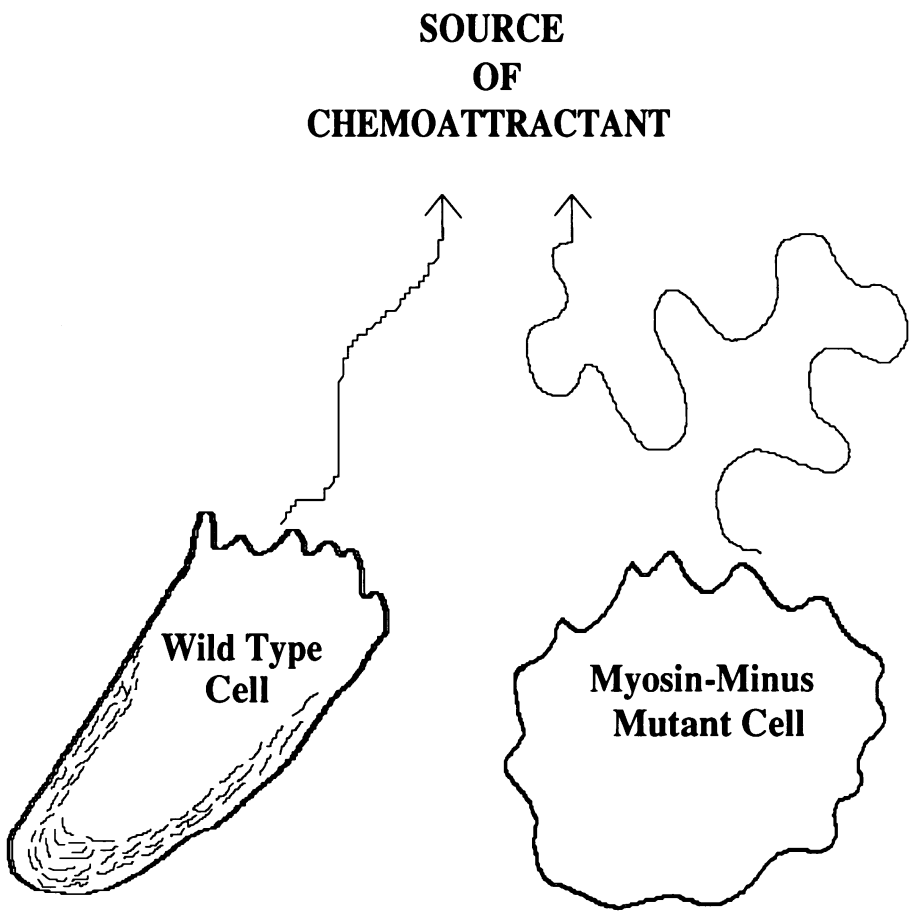

Figure 1. **Schematic diagram of a polarized *Dictyostelium* wild type cell with myosin filaments in its posterior region and a less polar myosin-minus mutant, both undergoing chemotaxis.** The myosin-minus cell moves a much greater total distance to reach the source (Wessels *et al.,* 1988), perhaps because of significant loss of its ability to become polar.

most of its conventional myosin in the posterior region (Yumura and Fukui, 1985), where there is very little membrane ruffling. In contrast, there is very little conventional myosin in the anterior portion of the cell, where filopodial and pseudopodial projections leading to ruffling are prevalent. The coincidence between the location of myosin in a wild type cell and the lack of ruffling in that region and the observation that myosin-minus cells demonstrate ruffling over their entire surface both suggest that *myosin may primarily inhibit pseudopodial projections* rather than act as a positive force in such projections.

Interestingly, translocations of myosin from the cortex to the endoplasm and possibly back to the cortex again have been shown to occur in response to the chemotactic stimulus (Yumura and Fukui, 1985; Berlot *et al.,* 1987; Liu and Newell, 1988). An important second message in the chemotactic response in *Dictyostelium* is cyclic GMP (cGMP), and Liu and Newell (1988) provide evidence from mutants defective in the cGMP-specific phosphodiesterase that this second message regulates the interaction of myosin with the cytoskeleton during chemotaxis.

A speculative but reasonable series of events, then, for eukaryotic chemotaxis is as follows: an unstimulated cell may be somewhat rounded because of a contractile shell of an actin-myosin network in the cortex (Figure 2, top, left). This network is postulated to inhibit events necessary for pseudopodial projections. For example, if membrane vesicle fusion with the cell membrane is involved (Bergmann *et al.,* 1983; Singer and Kupfer, 1986), the dense cortical actin-myosin network may present a barrier to that fusion. Stimulation of one edge of the cell with chemoattractant results in biochemical changes in the actin-myosin network that lead to local breakdown of perhaps both the actin filaments and the myosin thick filaments (Figure 2, middle, left). The barrier for pseudopod extension is, therefore, removed, and the cell extends toward the source of chemoattractant (Figure 2, bottom, left). The disassembled myosin may reassemble in the posterior portion of the cell, thus strengthening

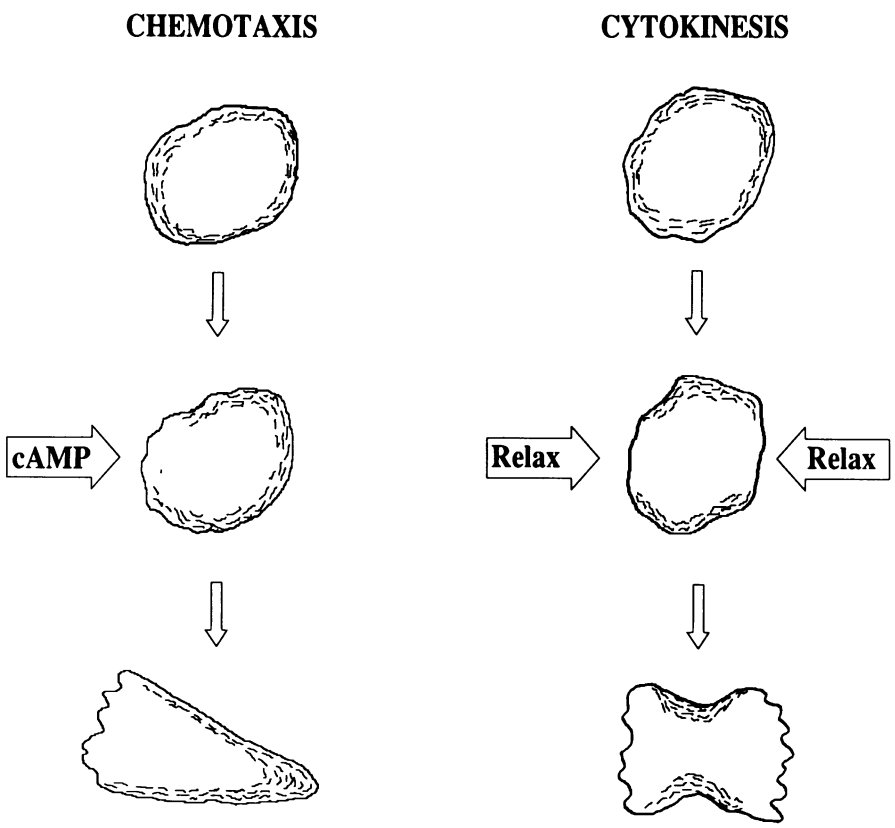

Figure 2. **A hypothetical drawing of nonpolarized cells that become polarized as a result of local breakdown of a cortical shell of myosin filaments.** Left, a *Dictyostelium* cell undergoing a chemoattractant-elicited response to cAMP. Right, a cell entering cytokinesis after relaxation at the poles, causing local breakdown of myosin filaments.

the existing cortical actin-myosin network there and providing further inhibition of membrane ruffling in that area. In this way, myosin may play a major role in the generation of cell polarity for efficient directed migration. The myosin in the posterior of the cell may, of course, simultaneously play a role as a positive force transducer, as suggested earlier, but the results with the myosin-minus mutants show that such a positive force transduction by this myosin is not required for anterior pseudopodia formation.

These concepts also can be applied to a cell initiating cytokinesis (Figure 2, right). In this case, some internal polar signal, probably involving the asters of the mitotic apparatus, may cause the same type of breakdown of the cortical contractile apparatus, with the myosin being relocalized to the furrow region for cytokinesis. This would result in polar relaxation, a concept promoted by Bray and White (1988), and, analogous to the unipolar ruffling of the chemotaxing cell, in ruffling of the cell membrane at both poles of the dividing cell.

A suggested role of myosin heavy chain phosphorylation in polarity generation. If one looks for chemical modifications that might affect *Dictyostelium* myosin filament disassembly and reassembly in vivo, phosphorylation of the tail portion of the myosin is the most likely candidate. Myosin heavy chain phosphorylation occurs on the tail portion of the molecule on several threonine residues (Peltz *et al.*, 1981; Claviez *et al.*, 1982; Pagh *et al.*, 1984) and inhibits myosin thick-filament formation in vitro (Kuczmarski and Spudich, 1980; Ravid and Spudich, 1989). This phosphorylation may inhibit filament assembly by inducing the formation of a bent monomer of myosin whose assembly domain is tied up in an intramolecular interaction that precludes intermolecular interactions which are necessary for thick filament formation (Pasternak *et al.*, 1989a).

Is this myosin tail phosphorylation physiologically relevant? The first correlation of myosin phosphorylation with *Dictyostelium* chemotaxis derived from studies of Rahmsdorf *et al.* (1978) and Malchow *et al.* (1981). They showed that there is a transient increase in the rate of myosin heavy-chain phosphorylation in extracts made from cells at various times after cAMP stimulation of intact cells. These studies were followed by experiments of Berlot *et al.* (1985; 1987), who labeled *Dictyostelium* cells to high specific radioactivity with $^{32}P_i$ and performed kinetic analyses

of myosin phosphorylation in vivo during a chemoattractant-elicited response. In vivo experiments of this type showed transient increases in myosin heavy chain as well as light chain phosphorylation, and the kinetics of the phosphorylation changes fit well the known shape changes associated with the chemotactic response (Fontana *et al.,* 1986). Dose-response curves for the extent of myosin phosphorylation as a function of chemoattractant concentration are very similar to the cAMP concentration dependence of the chemotactic response of the intact cells (Van Haastert, 1983). Furthermore, the adaptation response of the phosphorylation changes parallel those of the chemotactic response of the cells.

These results strongly suggest that the cAMP-induced myosin phosphorylation responses are part of the chemotactic sensing mechanism. Our working hypothesis is that these phosphorylation changes are involved in the redistribution of the myosin and that this redistribution is important for generation of cell polarity.

Cells probably contain multiple actin-based motors, which may derive from fusing the S-1 motor unit to a variety of types of tail

The observations that myosin-minus cells undergo a variety of forms of movement, including ruffling of membranes and cell migration, have prompted speculation that microtubule-based motors, as well as other actin-based motors, may drive these motile processes. A fascinating recent realization is that in vivo the myosin molecular motor unit (Toyoshima *et al.,* 1987), S-1, is probably attached to a variety of carboxy-terminal tails that possibly determine which particular cellular process that motor unit has been commandeered to drive (Figure 3). Thus, for events such as cytokinesis, capping of surface receptors, and changes in shape associated with morphogenesis, an α-helical coiled-coil tail with self-assembly properties may be essential so that a bipolar filament can form. The bipolar filament provides the opportunity to pull on actin filaments from opposite directions, resulting in two points in the cell being drawn closer together (Figure 4, top). In other cases, the tail of the actin-based motor may associate with other elements in the cell, such as vesicles, organelles, or filaments, which can then be drawn along actin tracks for productive relocation within the cell (Figure 4, bottom).

The above concept has grown out of the discovery by Pollard and Korn (1973) that *Acanthamoeba* contains a small unusual actin-based motor called myosin I, which has been shown to generate movement in vitro (Albanesi *et al.,* 1985). This unusual actin-based motor from *Acanthamoeba* has been characterized extensively by Pollard and his coworkers and by Korn and his colleagues (for review, see Korn and Hammer, 1988). Myosin I has been immunolocalized at or near the plasma membrane of both *Acanthamoeba* and *Dictyostelium* (Gadasi and Korn, 1980; Fukui *et al.,* 1989). Adams and Pollard (1986 demonstrated myosin I dependent movement in vitro of membranes isolated from *Acanthamoeba,* and more recently they showed that myosin I binds directly to membranes and lipids (Adams and Pollard, 1989). Thus, myosin I may be associated with membranes in cells and involved in their movements. The discovery of proteins similar to *Acanthamoeba* myosin I in *Dictyostelium* (Cote *et al.,* 1985) and in the brush border of mammalian epithelia (Collins and Borysenko, 1984), where it is associated with a membrane fraction (Mooseker *et al.,* 1989), indicate that this molecule

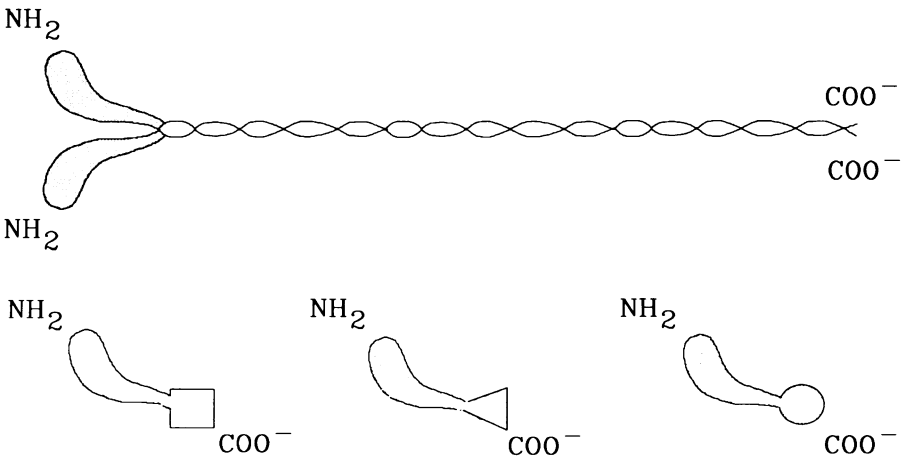

Figure 3. **Schematic diagram of myosin and other putative actin-based motors.** Whereas myosin is made from two heavy chains that interact to form an α-helical coiled-coil tail, which is involved in thick filament formation, the other actin-based motors have similar motor units (the amino-terminal portion of the protein) but different tail moieties (the carboxy-terminal portion of the protein) depicted by the square, triangle, and circle, respectively.

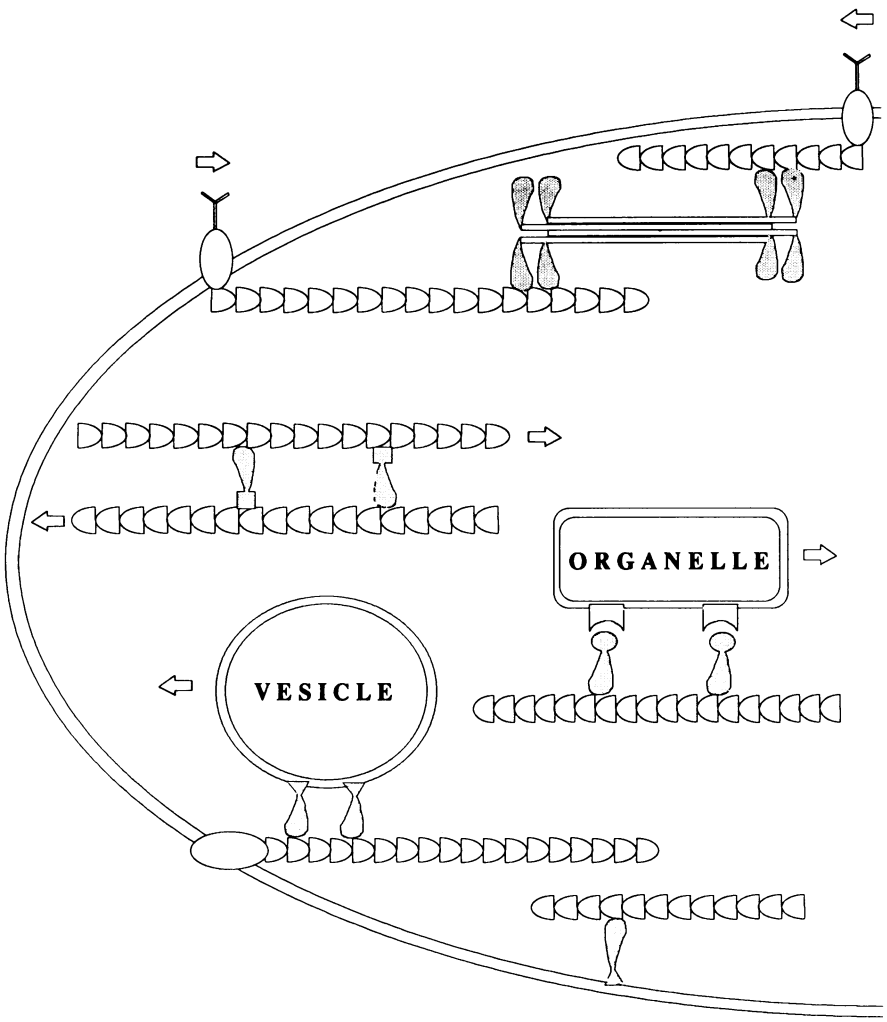

Figure 4. **A speculative schematic diagram of various types of actin-based motors carrying out different functions within a cell.** The actin filaments are represented as simplified polar structures. Conventional myosin forms bipolar filaments that can pull two points in the cell together. At the top, for example, a myosin thick filament is participating in capping of ligand-bound receptor molecules. The tail of some actin-based motors may bind directly to actin filaments (Lynch *et al.,* 1986) and, for example, cause shear between different filaments (middle). Another type of tail may interact with an appropriate receptor, for example, on an organelle surface, to cause movement of that organelle along actin filaments within the cytoplasm. Other tails may have a domain for direct binding to lipids (Adams and Pollard, 1989) and bind directly to vesicles or membranous organelles for their movement along actin, for example, to be brought into position to fuse with the plasma membrane (bottom). Such fusion would result in that actin-based motor becoming part of the plasma membrane, where it may interact with cortical actin.

probably exists in all eukaryotic cells, where it might serve essential functions in cell movement or cell shape changes.

Jung *et al.* (1987; 1989) have sequenced from both *Acanthamoeba* and *Dictyostelium* an actin-based motor gene reported to correspond to the myosin I protein described earlier. The deduced amino-acid sequence of the head is highly conserved with that of conventional myosin, while the tail is unusual and extremely different from that of conventional myosin. The myosin I tail consists of two domains: the carboxy-terminal half demonstrates ATP-insensitive binding to actin (Lynch *et al.,* 1986) while the amino-terminal half binds to membranes and pure phospholipid vesicles (Adams and Pollard, 1989).

In *Dictyostelium,* there is only one conventional myosin gene, *mhc*A, but there are a number of different actin-based motor genes, all of which are transcribed (Jung *et al.,* 1989; Titus *et al.,* 1989). *Dictyostelium* is the organism of choice for addressing the roles of these actin-based motors in vivo, because of the advances in the application of molecular genetic approaches in this organism, the ease with which biochemical and other manipulations are carried out, and the similarities in the behaviors of this cell with cells of higher organisms. Jung *et al.* (1989) isolated a *Dictyostelium* actin-based motor gene with the use of an antibody prepared against *Dictyostelium* myosin I. They showed that this gene is a single-copy gene by the use of a DNA probe directed to the unusual region that corresponds to the myosin I tail. Thus they isolated one particular actin-based motor gene. In contrast, Titus *et al.* (1989) undertook a broad search for genes encoding actin-based motors in *Dictyostelium* with the use of a probe from the highly conserved re-

gion of the myosin head. This region would be expected to be important for motor function and therefore present in all actin-based motor molecules. Using this approach, they have shown that there are several actin-based motor genes in *Dictyostellium.* The sequence of one of these, *abm*A, is 50% homologous to the *Dictyostelium* myosin I in the S-1 region. In contrast, the tail regions of *abm*A and myosin I are only 30% homologous, and the putative ATP-insensitive actin-binding sequence is not present in the deduced *abm*A gene product but a putative membrane-binding sequence is. The lack of conservation of the tail sequences may indicate that these two actin-based motors perform distinct functions within the cell.

Future perspectives

It seems likely that the manifestation of the multiple forms of movement apparent in a eukaryotic cell derives from multiple motors. The cell may have achieved this diversity by linking the myosin molecular motor unit to a wide variety of tails, so as to use this mechanochemical enzyme moiety for a variety of purposes. The tail would then specify the particular function of that actin-based motor. It seems likely that there will be a whole spectrum of such motors, the *nina*C gene from *Drosophila* (Montell and Rubin, 1988) being one extreme case. One may therefore expect that biochemists will discover a number of new actin-based motors over the next several years. If so, it will be exciting to use molecular genetics to disrupt or delete their respective genes and to examine the resulting phenotypes of the mutant cells. *Dictyostelium* is an excellent organism for such a molecular dissection of cell functions and should prove equally valuable in the elucidation of the roles of the microtubule-based motors. The next decade should see the definition in molecular terms of each of the bewildering forms of cell motility characteristic of eukaryotic cells.

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