Journal of Cell Science 108, 387-393 (1995)
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# A mechanical function of myosin II in cell motility

**Patrick Y. Jay, Peter A. Pham, Scott A. Wong and Elliot L. Elson***

Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

*Author for correspondence

## SUMMARY

**Myosin II mutant *Dictyostelium* amoebae crawl more slowly than wild-type cells. Thus, myosin II must contribute to amoeboid locomotion. We propose that contractile forces generated by myosin II help the cell's rear edge to detach from the substratum and retract, allowing the cell to continue forward. To test this hypothesis, we measured the speed of wild-type and myosin II null mutant *Dictyostelium* cells on surfaces of varying adhesivity. As substratum adhesivity increased, the speed of myosin II null mutant cells decreased substantially compared to wild-type cells, suggesting that the mutant is less able to retract from sticky surfaces. Furthermore, interference reflection microscopy revealed a myosin-II-dependent contraction in wild-type but not null mutant cells that is consistent with a balance of adhesive and contractile forces in retraction. Although myosin II null mutant cells have a defect in retraction, pseudopod extension does not cause the cells to become elongated on sticky surfaces. This suggests a mechanism, based possibly on cytoskeletal tension, for regulating cell shape in locomotion. The tension would result from the transmission of tractional forces through the cytoskeletal network, providing the myosin II null mutant with a limited means of retraction and cell division on a surface.**

Key words: cell motility, adhesion, cytoskeleton, myosin II, *Dictyostelium*

## INTRODUCTION

Mutant *Dictyostelium* that lack myosin II crawl more slowly than their parental wild-type strains (Wessels et al., 1988). This indicates that myosin II contributes to locomotion but does not suggest how. To identify a specific function of myosin II or any other cytoskeletal protein, one can consider the basic steps required for amoeboid locomotion and then devise experiments that tax the mutant's ability to perform each step. A cell begins to crawl by extending a pseudopod that forms adhesive contacts with the substratum. Next, the cell exerts traction against these contacts. Selective detachment of the more posterior adhesive contacts allows this traction to produce forward motion. A cell cannot crawl on a substratum that is either too slippery or too sticky because it either cannot exert sufficient traction or cannot detach itself, respectively. A surface of optimum adhesiveness between these two extremes will support maximal motility (DiMilla et al., 1991). Smooth muscle cells migrating on fibronectin or collagen, neural crest cells on anti-$\beta_1$ integrin antibodies, and myoblasts on laminin, exhibit this kind of dependence of crawling speed on substratum adhesivity (DiMilla et al., 1993; Duband et al., 1991; Goodman et al., 1989).

Myosin II can produce a cortical tension that causes *Dictyostelium* cells to round up and stiffen. This occurs, for example, as a result of the 'rigor' response in cells depleted of ATP by sodium azide (Pasternak et al., 1989). Wild-type cells treated with azide also detach from the coverslip, but myosin II mutant cells do not round up, stiffen or detach (Pasternak et al., 1989; unpublished observations). Thus, the tension generated by myosin II can detach a cell from its substratum adhesion sites. Surface particle tracking measurements on wild-type (Ax2) and myosin II null mutant (mhcA-) *Dictyostelium* cells suggest that a myosin-II-based force predominates at the posterior of the cell (Jay and Elson, 1992). This conclusion is supported by mechanical measurements and morphological data (Pasternak and Elson, 1990; Yumura et al., 1984). These data led us to put forward the hypothesis that one specific mechanical contribution of myosin II to locomotion is to provide a contractile force that detaches the cell from its posterior adhesion sites. This concept had earlier been proposed for both fibroblasts and *Dictyostelium* (Chen, 1981; Small, 1989).

Bell's (1978) theory of cellular adhesion predicts that a mechanical force acting against a bond can increase the dissociation rate of a receptor and the bound ligand. Recent experiments support this theory and demonstrate the relationship between the mechanical strength of adhesion and the number and chemical affinity of receptor-ligand bonds (Kuo and Lauffenburger, 1993). Thus, as a cell crawls forward, its ability to detach from its posterior contacts may depend in part on the contractile force exerted against the bonds between the plasma membrane and substratum. If myosin II promotes tail retraction, this specific mechanical function should be apparent in

motility assays of wild-type and myosin II null mutant amoebae on surfaces of varying adhesivity. As the stickiness of a surface increases, the speed of mhcA$^-$ cells should decrease more than that of the wild type because the mutant lacks the force of myosin II to break the increased number of posterior contacts. We tested this prediction by measuring the locomotion of Ax2 and mhcA$^-$ cells on glass coverslips coated with different amounts of polylysine to produce substrata of varying adhesivities.

## MATERIALS AND METHODS

### Cell cultures and preparation

Wild-type (Ax2) and myosin II null mutant (mhcA$^-$) *Dictyostelium* were gifts from the laboratory of Dr James Spudich. The mhcA$^-$ cells were made by deletion of the myosin II heavy chain gene through homologous recombination (Manstein et al., 1989). The cells were grown in HL5 medium. For the experiments the cells were harvested, washed, and starved for seven hours on filter pads soaked with buffer (40 mM potassium phosphate, pH 6.4, 20 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$).

### Coverslip preparation and adhesivity assays

The adhesiveness of coverslips was varied by coating them with polylysine in concentrations ranging from 0 to 30 mg/ml. A 1, 10 or 30 mg/ml polylysine solution (molecular mass >400 kDa, Sigma, St Louis, Missouri) was spread on one face of an acid-washed glass coverslip (50 μl/cm$^2$). The coverslips were rinsed thoroughly and dried after coating with the polylysine solution for four hours. The relative adhesivities of polylysine-coated and uncoated coverslips were determined in a centrifugation assay. Ax2 and mhcA$^-$ cells were allowed to settle for ten minutes on uncoated or coated coverslips (25 mm diameter). The coverslips were subsequently placed upside-down in a 50 ml conical tube filled with buffer. A washer wedged at the top of the tube held the coverslip in place. The tubes were spun in a centrifuge at 2500 ***g*** for ten minutes; the centrifugal force caused cells to detach and fall to the bottom of the tube. Higher-speed spins tended to break the coverslips. The number of cells remaining on the coverslips was counted in ten randomly selected microscopic fields. The number of cells on a set of control coverslips that were not spun was also counted. The measurements were done in triplicate.

### Motility and morphology assays

Starved cells were gently rinsed off the filter pads and allowed to settle on coverslips for ten minutes before their motility or morphology was recorded by video microscopy. The assays were done in Sykes-Moore chambers (Bellco Glass, Vineland, New Jersey) perfused with buffer at 4 ml/min. For the motility assays the cells were observed with a ×16 phase-contrast objective. The speed of every cell in a 390×280 μm video field was obtained by manually tracking the centroids every minute for approximately 15 minutes using software written by Dr Scot Kuo and Jit Tan for the Imaging Technology Series 150 image processor. The speed of a cell is calculated as its average displacement per one-minute interval. This definition of cell speed was chosen to maintain consistency with previous data (Wessels et al., 1988).

For the morphology experiments a ×40 phase-contrast objective was used to scan cells randomly. Images of cells were recorded for subsequent length, circumference and area measurements with an Argus 10 image processor (Hamamatsu, Japan). Length was measured along the anterior-posterior axis of a cell. Roundness, $r$, was determined by the equation, $r = 4\pi \times \text{area}/\text{circumference}^2$; thus, $r$=1 for a circle and approaches zero as a cell becomes longer and narrower.

### Interference reflection microscopy

IRM images of cells on uncoated or 30 mg/ml polylysine-coated coverslips were obtained as described (Gingell and Vince, 1982; Heuser et al., 1993). The images were obtained using monochromatic (546 nm) epi-illumination and a Leitz ×63 objective with a numerical aperture of 1.3. Video images were recorded with a Hamamatsu C2400 SIT camera onto a Panasonic TQ3038F optical memory disk recorder.

## RESULTS

### *Dictyostelium* motility on surfaces of varying adhesivity

The adhesiveness of glass coverslips was varied by coating them with polylysine. When subjected to a 2500 ***g*** centrifugal force, the number of cells that detached from coverslips depended on the polylysine-coating concentration (Fig. 1). Coverslips coated with 30 mg/ml polylysine showed greater than 100% adherence, probably because some cells detached from the control, non-centrifuged coverslips during handling. Ax2 and mhcA$^-$ cells are equally adherent to the coverslips under the conditions of this assay.

The speeds of Ax2 and mhcA$^-$ cells on uncoated coverslips agree with the published values for wild-type *Dictyostelium* and a truncated, non-functional myosin II mutant, respectively (Wessels et al., 1988). The speed of Ax2 cells is the same on uncoated and 10 mg/ml polylysine-coated coverslips and decreases slightly on 30 mg/ml polylysine-coated coverslips (Fig. 2). On coverslips coated with ≥10 mg/ml polylysine the speed of mhcA$^-$ cells decreases substantially from their speed on uncoated coverslips (Fig. 2).

The calculated speed of mhcA$^-$ cells on ≥10 mg/ml polylysine-coated coverslips is approximately one-third of that seen on uncoated coverslips, but time-lapse videos show that mhcA$^-$

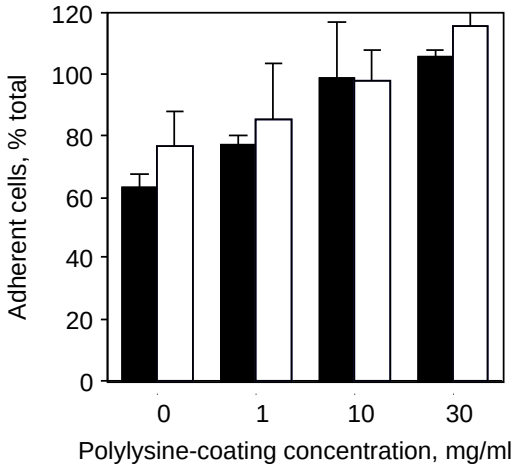


**Fig. 1.** The adhesivity of *Dictyostelium* cells for coverslips varies with the polylysine-coating concentration. To quantify the adhesivity of a coverslip, adherent cells were subjected to a 2,500 ***g*** centrifugal force, causing them to detach and fall to the bottom of a tube. The remaining cells were counted in randomly selected microscopic fields. Adhesivity is expressed as the percentage of cells on a coverslip as compared to control, non-centrifuged coverslips. Ax2, filled bars; mhcA$^-$, open bars. $n$=3. Error bars represent s.e.m.

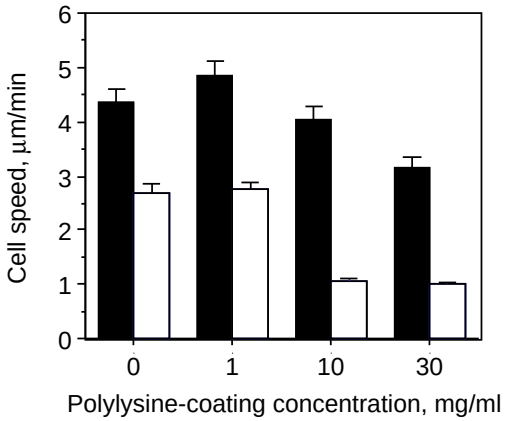


**Fig. 2.** The motility of Ax2 and mhcA⁻ cells on substrata of varying adhesivity. The average speed of Ax2 (filled bars) and mhcA⁻ cells (open bars) on coverslips coated with varying concentrations of polylysine. Each data point represents the average speed of 34-52 cells on two different coverslips. Error bars represent s.e.m.

cells are to a large extent stuck in place on more adhesive coverslips. In Fig. 3, the position of every cell in the video field of a representative experiment is plotted at one-minute intervals for Ax2 and mhcA⁻ cells on uncoated and 10 mg/ml coated coverslips. The length of a typical *Dictyostelium* cell is about 15 μm. As illustrated by the trajectories, Ax2 cells translocate effectively on an adhesive substratum and traverse up to several cell lengths during a 15 minute experiment. Most mhcA⁻ cells wobble in place around stationary points of adhesive contact with the substratum. Four of the 25 mhcA⁻ cells shown in Fig. 3D move only slightly more than 10 μm.

The apparent speed of mhcA⁻ cells that are stuck on coverslips results from its definition as the average displacement per one-minute interval. If the average displacement is measured over longer time intervals, the speed of stuck mhcA⁻ cells approaches zero while the moving Ax2 and mhcA⁻ cells have non-zero speeds, which only increases the significance of the differences in Fig. 2. Thus, on ≤1 mg/ml polylysine-coated coverslips the speed of mhcA⁻ cells is 55-60% of the wild type's when measured at either one- or three-minute intervals. But on 10 mg/ml polylysine-coated coverslips, the speed of mhcA⁻ cells is 26% of the wild type's at one-minute intervals and 17% at three-minute intervals. (The dependence of the apparent speed on the defined time interval has been treated in a theoretical discussion (Tourtellot et al., 1991).)

### Interference reflection microscopy of *Dictyostelium* cells on surfaces of varying adhesivity

Wild-type *Dictyostelium* cells on very adhesive substrata form wide, 'ultrathin lamellae' in which the dorsal and ventral membranes are separated by about 100 nm (Gingell and Vince, 1982). The apposition of the two membranes results in a dark

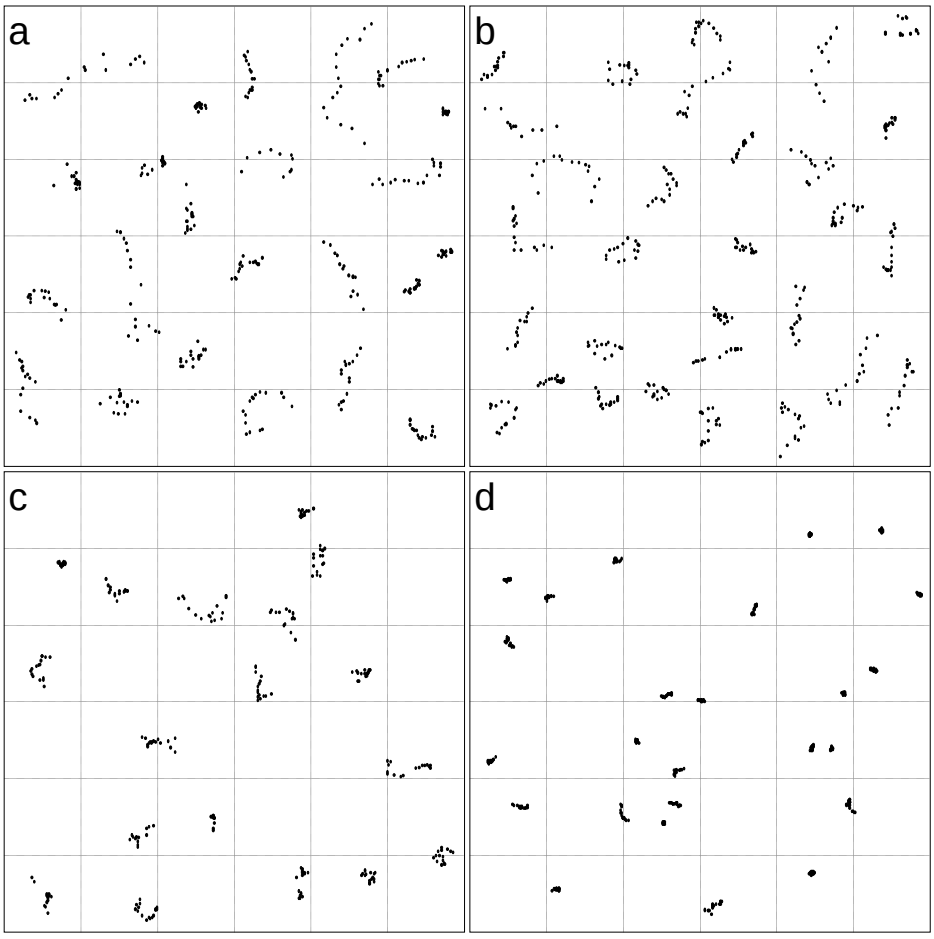


**Fig. 3.** Time-lapse videos show that mhcA⁻ cells appear stuck on coverslips coated with ≥10 mg/ml polylysine, whereas Ax2 cells are unaffected. The position of every cell in the video field of a given experiment is plotted at one-minute intervals to illustrate this point: Ax2 cells on an uncoated (a) and 10 mg/ml polylysine-coated coverslips (b); and mhcA⁻ on uncoated (c) and 10 mg/ml polylysine-coated coverslips (d). The trajectories of some cells have been separated from the others for clarity. One division, 50 μm.

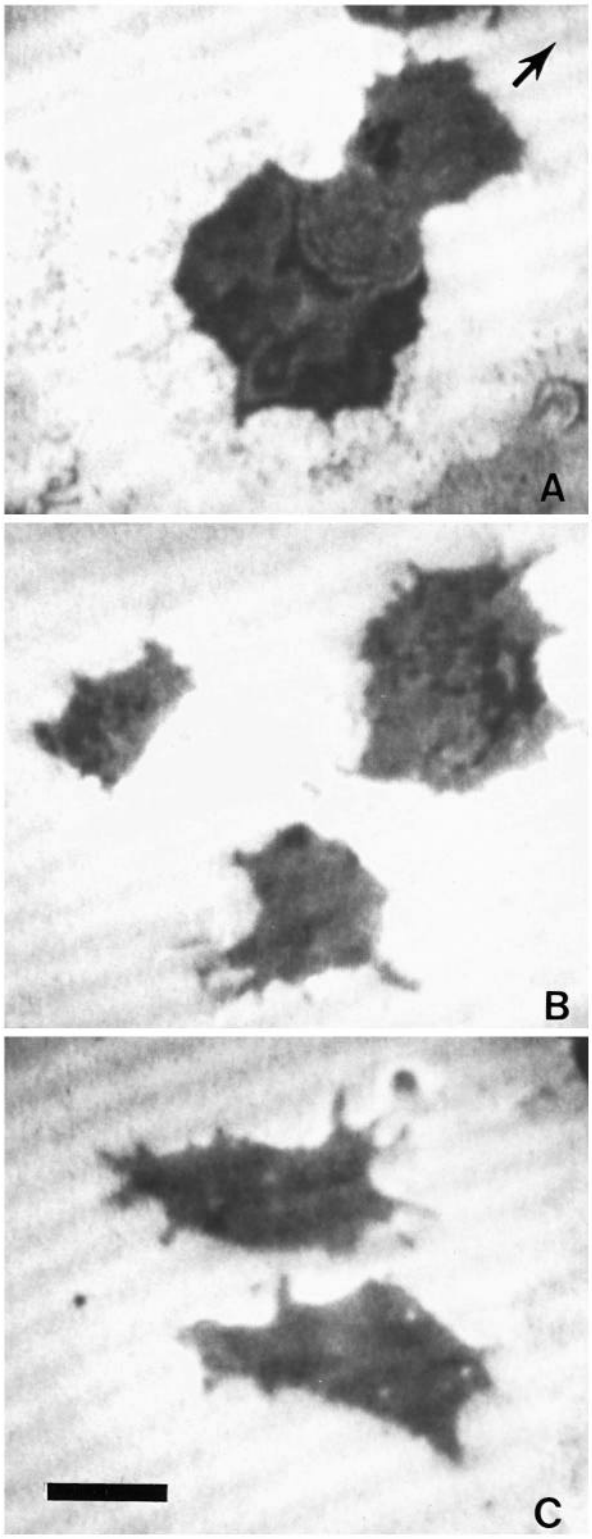


**Fig. 4.** Interference reflection microscopy of Ax2 and mhcA⁻ cells on uncoated and 30 mg/ml polylysine-coated coverslips. (A) The ultrathin lamella of an Ax2 cell on a polylysine-coated coverslip appears as a wide black region at the posterior of the cell. As the cell crawls forward, some debris is left behind the ultrathin lamella. The arrow indicates the direction of locomotion. Ax2 cells on uncoated coverslips (B) and mhcA⁻ cells on coated coverslips (C) do not form ultrathin lamellae. The darker portions of the IRM image represent the close apposition of either an organelle or the dorsal membrane with the ventral membrane. Bar, 5 μm.

region in the interference reflection microscopy (IRM) image of an Ax2 cell on a polylysine-coated coverslip (Fig. 4A). The lighter gray area results from reflections between the ventral membrane and substratum. (The interpretation of IRM images of *Dictyostelium* cells is discussed in greater detail by Gingell and colleagues (Gingell and Vince, 1982; Gingell et al., 1982).) Time-lapse observations show that ultrathin lamellae form at the posterior region of polarized or locomoting cells. Non-polarized cells, which lack a front as defined by the direction of locomotion or pseudopod activity, may have ultrathin lamellae circumferentially located. Ultrathin lamellae differ from leading edge lamellae because they never extend outward as pseudopods or leading lamellae do and are never seen at the front of a cell. They are instead associated with areas from which the cell is retracting. These observations are entirely consistent with those originally reported by Gingell and his colleagues.

Neither Ax2 cells on plain glass nor mhcA⁻ on plain or polylysine-coated coverslips form ultrathin lamellae. The IRM image of three Ax2 cells on uncoated glass shows only the gray reflection between the ventral membrane and the coverslip (Fig. 4B). The IRM observations of Ax2 cells on uncoated and polylysine-coated coverslips reproduce those reported for wild-type *Dictyostelium* (Gingell and Vince, 1982). The same experiments with mhcA⁻ cells show that ultrathin lamella formation requires myosin II. We were unable to find a single example among more than 500 mhcA⁻ cells on ten polylysine-coated coverslips in separate experiments. Fig. 4C shows a representative IRM image of two mhcA⁻ cells on a polylysine-coated coverslip.

### *Dictyostelium* morphology on surfaces of varying adhesivity

Amoeboid locomotion requires a cell to extend its leading edge and to retract its trailing edge. If extension operated independently of retraction, mhcA⁻ cells that are stuck on polylysine-coated coverslips should elongate to a greater extent than wild-type cells. Both Ax2 and mhcA⁻ cells, however, maintain a constant average length over a range of substratum adhesivities (Table 1). Cell shape, assessed quantitatively by the roundness of cells and qualitatively by eye, is also independent of substratum adhesivity (Table 1). As previously reported, mhcA⁻ cells are rounder than the wild type ($P<0.01$ on all coverslips by two-tailed *t*-test; Wessels et al., 1988).

## DISCUSSION

Myosin II mutant *Dictyostelium* cells crawl about half as fast as the wild type on plain glass surfaces (this work; Wessels et al., 1988). Hence, myosin II is not essential for locomotion on glass, but it does contribute. The experiments presented in this paper test the hypothesis of a specific mechanical function for myosin II, that of promoting detachment of the posterior of the cell from the substratum. Myosin II may serve other locomotor functions as well. For example, myosin II could inhibit the ectopic extension of pseudopodia by stiffening the cell

**Table 1. The length and roundness of *Dictyostelium* cells on surfaces of varying adhesivity**

| Polylysine concentration (mg/ml) | Length (μm) | | Roundness (*r*) | |
|---|---|---|---|---|
| | Ax2 | mhcA⁻ | Ax2 | mhcA⁻ |
| 0 | 14.9±3.0 | 13.6±3.2 | 0.36±0.08 | 0.46±0.11 |
| 1 | 14.5±3.1 | 13.6±3.2 | 0.41±0.10 | 0.48±0.12 |
| 10 | 14.5±2.6 | 13.5±3.1 | 0.37±0.12 | 0.46±0.14 |
| 30 | 14.5±2.3 | 12.6±2.6 | 0.41±0.11 | 0.46±0.11 |

The length and roundness of Ax2 and mhcA⁻ cells are independent of substratum adhesivity and thus crawling speed. Cells do not elongate as substratum adhesivity increases and speed subsequently decreases. Each data point represents the mean and standard deviation of 60 cells on two coverslips.

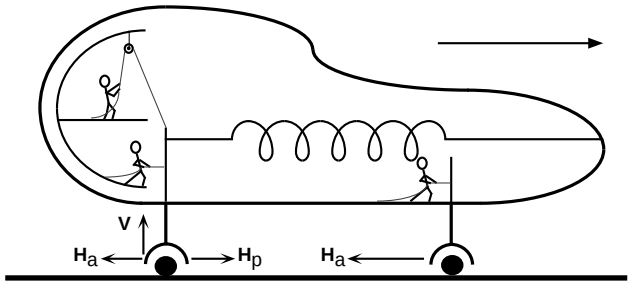


**Fig. 5.** In this schematic model of motility the cell is crawling to the right. The homunculi, representing myosin-II-dependent and -independent mechanisms, provide the forces for traction and retraction. Those forces act on anterior and posterior bonds to the substratum and are depicted by the vectors. The tractional force, $\mathbf{H}_a$, is horizontal and antiparallel to the direction of locomotion. When exerted against an immobile link to the substratum, $\mathbf{H}_a$ will move the cell forward. The molecular basis of $\mathbf{H}_a$ at the front of the cell is unknown, but the force unquestionably exists according to Newton's laws. At the back of the cell myosin II exerts vertical and horizontal forces, **V** and $\mathbf{H}_a$, that promote detachment of bonds to the substratum. The myosin-II-based $\mathbf{H}_a$ may also support traction. As the front of the cell moves forward, cytoskeletal tension, indicated by the spring, exerts a horizontal force parallel to the direction of locomotion on the posterior bond ($\mathbf{H}_p$). $\mathbf{H}_p$ is a force that could promote detachment in mhcA⁻ cells.

posterior, thereby promoting continuous forward motion (Spudich, 1989). Other specifically designed experiments would be needed to test this possibility.

As predicted by our hypothesis, the importance of myosin II for locomotion increases with the adhesivity of the substratum. On coverslips coated with a 10 mg/ml polylysine solution the motility of wild-type Ax2 cells is no different from that on uncoated glass. Furthermore, the speed of Ax2 cells diminishes only slightly on 30 mg/ml polylysine-coated coverslips. In contrast, mhcA⁻ cells cannot translocate on ≥10 mg/ml polylysine-coated coverslips over the time period of our observations. Hence, on these substrata myosin II is essential because it overcomes adhesive forces in locomotion.

The role of myosin II in promoting detachment can be understood in terms of the rate of dissociation of receptor-ligand bonds between the cell and substratum. Bell theorized that the dissociation rate could be increased by a mechanical force acting against the bond (Bell, 1978). Measurements of the force required to detach an antibody-coated bead from a staphylococcal Protein A-coated surface under conditions of varying antibody concentrations or affinities support Bell's model (Kuo and Lauffenburger, 1993). A cytoskeletal force may act on a bond via direct or indirect interactions with the membrane receptor. The force may be exerted against a bond vertically or parallel or antiparallel to the direction of locomotion. The possible forces acting on two bonds under the front and rear of the cell are shown in Fig. 5. According to Newton's third law, these forces will act on the cell in the opposite direction.

The enhanced velocity of bead transport and the differential stiffening toward the rear of wild-type relative to mhcA⁻ cells suggest that myosin-II-dependent contractile forces are generated largely at the posterior of the cell (Jay and Elson, 1992; Pasternak and Elson, 1990). Observation of the formation of ultrathin lamellae by wild-type *Dictyostelium* on highly adhesive surfaces confirms this suggestion. These lamellae, in which the dorsal and ventral membranes are separated by about 100 nm, were originally described by Gingell and his colleagues using interference reflection microscopy. They showed that formation of ultrathin lamellae is temperature-dependent and occurs at the posterior of polarized cells (Gingell and Vince, 1982). In an IRM image an ultrathin lamella is darker than the cell body, but it is not in closer contact with the coverslip than the rest of the cell. The entire cell, including the ultrathin lamella, is separated from the coverslip by a uniform distance, as determined by total internal reflection fluorescence (Todd et al., 1988). Repeating these experiments with wild-type and mhcA⁻ cells, we find that ultrathin lamella formation requires myosin II.

Both vertically and horizontally oriented retraction forces that are resisted by an adhesive substratum could cause the formation of ultrathin lamellae. One simple model supposes that myosin II in the cytoskeletal cortex of the tail generates an isotropic contraction similar to the global cortical tension that develops during capping (Bray and White, 1988; Pasternak et al., 1989). As shown in Fig. 5, ultrathin lamellae could be formed by a vertical component of the myosin-II-based force, symbolized by the homunculus and pulley pulling the dorsal membrane down to the adherent ventral surface. Alternatively, a horizontal component of the myosin-II-based force could draw the cytoplasm forward from a firmly anchored tail and cause thinning of the rear edge. Either vertical or horizontal components would promote detachment via cytoskeletal-membrane interactions as well as formation of ultrathin lamellae. Ax2 cells on uncoated glass and mhcA⁻ cells on uncoated or coated surfaces do not form ultrathin lamellae because they, respectively, lack the balancing adhesive force or the myosin II-dependent retraction force. The assay for ultrathin lamella formation tests a cell's ability to transduce a myosin-II-based force through the cell membrane to the substratum or, in essence, to retract. One might therefore predict the existence of other types of mutants that could not form ultrathin lamellae. For example, a mutant that lacked the necessary membrane-cytoskeletal linkage proteins would be unable to transmit force to the adhesion receptors.

The velocity of rearward bead transport is faster towards the rear of wild-type cells than on the same region of mhcA⁻ amoebae (Jay and Elson, 1992). Thus, a horizontal component

of the myosin II contractile force is directed rearward (Fig. 5). Like the vertical component, this force can destabilize cell-substratum bonds, but it also pulls the rear edge of the cell forward, as suggested in studies of fibroblast tail retraction (Chen, 1979). This highlights the distinction between the retractional and tractional forces. A retractional force, which decreases the lifetime of a bond between the cell and substratum, can be oriented horizontally or vertically. By definition, a net tractional force acts antiparallel to the direction of locomotion. To pull a cell forward, traction requires sufficiently strong anchorage of the anterior portion of the cell (DiMilla et al., 1991). Myosin II would have nothing to pull against if the substratum were too slippery. Therefore, the speed of wild-type cells should increase more rapidly than mhcA$^-$ cells as the adhesiveness of the substratum increases from very slippery conditions. We have not observed this differential increase in crawling speeds, but that does not exclude a role for myosin II in exerting traction. The absence of an increase in the speeds of Ax2 and mhcA$^-$ cells over the range of polylysine concentrations that we measured suggests that the optimum adhesiveness for *Dictyostelium* motility is equal to or less than that of clean glass coverslips.

Our observations show that myosin II contributes to cellular detachment and retraction. How then can the mhcA$^-$ cells crawl on plain glass to which they are substantially adherent (cf. Fig. 1)? Bead transport experiments suggest that the tractional force is greatest at the front of a *Dictyostelium* cell (Jay and Elson, 1992). The molecular basis of myosin-II-independent traction is still unknown, but any rearward force exerted on a fixed, substratum-bound particle will cause the cell to move forward (Fig. 5). We propose that cytoskeletal tension is generated by myosin-II-independent traction at the front of the cell. Represented by the spring in Fig. 5, the tension transmits a horizontal force parallel to the direction of locomotion to promote detachment at the rear of the cell. The indirect contribution of a tractional force to detachment represents a second example in which traction has been substituted for a myosin-II-based function. In the first such example, myosin II mutant cells, which have a cytokinesis defect, were able to divide when plated on a surface. Mutant daughter cells crawled away and separated from each other by traction-mediated cytofission (Fukui et al., 1990). Implicit in the observation is the existence of cytoskeletal tension that allows the cells to divide. Were mhcA$^-$ cells infinitely elastic, the daughter cells would elongate cylindrically but not separate as they crawled in opposite directions.

Tractional tension can help detach the tail from the substratum. This concept is best illustrated by fibroblasts in which the processes of extension, tractional exertion and retraction occur separately. As a fibroblast crawls forward, it stretches out until its tail snaps forward. Both elastic recoil and active contraction contribute to fibroblast tail retraction (Chen, 1981). The elastic recoil results from the tension of the cytoskeletal lattice created by forward extension. Myosin-II-independent tractional forces transmitting tension through a cytoskeletal network could provide mhcA$^-$ amoebae with an alternative but limited means for retraction. The relative contribution of passive, transmitted tension or active myosin-II-based contraction to retraction depends on the substratum, as shown in the current experiments, and may depend on the cell type. For example, nematode sperm, which have no myosin or other known contractile motor protein, crawl rapidly on clean, uncoated glass. When placed on adhesive substrata, however, the sperm become stuck in place, just as mhcA$^-$ mutant *Dictyostelium* cells do (Nelson et al., 1982; Sepsenwol and Taft, 1990). Passive tension generated by forward spreading appears to be the sole force driving retraction in nematode sperm and is sufficient only when the sperm does not form tight bonds with the substratum. Wild-type *Dictyostelium* may employ a combination of myosin-II-independent and -dependent retraction.

On highly adhesive surfaces, cytoskeletal tension could also prevent myosin II null mutant cells from stretching out, as one might have expected from unopposed extension at the front of the cell and a defect in retraction at the rear. Wild-type and mhcA$^-$ *Dictyostelium* cells maintain their size and shape within prescribed limits regardless of substratum adhesivity. Regulation of protrusive activity by cytoskeletal tension appears to be a general phenomenon of crawling cells. For example, when the tail of a migrating fibroblast detaches, transiently reducing cortical tension, lamellar activity and spreading increase and continue until the cell returns to its fully extended shape, in which the restored tension suppresses ruffling and spreading (Chen, 1979). In another example, increasing the tension in a fish epidermal keratocyte parallel to the direction of locomotion with a microneedle suppressed lateral pseudopod formation. Lamellar extension at the front may also have decreased, but this was not quantified (Kolega, 1986).

If cytoskeletal tension is transmitted throughout the cell, horizontal myosin-II-dependent and -independent retraction forces would require that a cell be able to stabilize anterior or destabilize posterior contacts selectively (DiMilla et al., 1991). Otherwise, cells would detach at the front or rear with equal probability and make no systematic progress in any direction, which is contrary to their observed behavior. The observation that the probability of attachment of membrane glycoproteins to the cytoskeleton is greatest at the leading edge of fish keratocytes provides one example of the stabilization of anterior contacts (Kucik et al., 1991). At the other end of the cell, neutrophils have been shown to reduce the affinity of their receptor for vitronectin by a $Ca^{2+}$/calcineurin-dependent mechanism (Hendey et al., 1992). We note that the vertically oriented myosin-II-based contractile force does not require selective stabilization or destabilization of bonds because it is exerted predominantly at the rear of the cell.

In summary, as substratum adhesivity increases, a crawling cell will form more or stronger bonds to the substratum that must be broken to continue forward. We observe that by increasing the adhesivity of glass coverslips the locomotion of *Dictyostelium* amoebae lacking myosin II is more retarded than that of wild-type cells. This experiment indicates that one function of myosin II is to enable forward progress by detaching the cell from its posterior contact with the substratum. In addition, the formation of ultrathin lamellae shows that the force of myosin II is exerted preferentially at the rear of the cell, consistent with its intracellular localization (Yumura et al., 1984). Previous work suggested that myosin II was dispensable for locomotion on plain glass (Wessels et al., 1988). In contrast, myosin II is essential for locomotion on more adhesive substrata. This emphasizes the fact that strong phenotypes for cytoskeletal mutants may be elicited only under conditions designed to stress the specific function of the

mutated protein. Cytoskeletal tension, which results from the balance of forces between anterior extension and posterior adhesion, provides an alternative mechanism for the mhcA$^-$ mutant to retract on surfaces of lower adhesivity. The tension may also maintain cell shape during locomotion and permit traction-mediated cytofission.

We thank Dr James Spudich and his laboratory for the cells. We are grateful to Dr John Heuser and John H. Morisaki for their insightful suggestions and the use of their microscope, and to Youngmee Park for her assistance in cell tracking. We also thank Drs Paul Bridgman, Chris Cheney, John Cooper, Carl Frieden and Dorothy Schafer for their comments on the manuscript. This work is supported by a grant from the NIH to E.L.E.

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(Received 9 August 1994 - Accepted 23 September 1994)