Relative Distribution of Actin, Myosin I, and Myosin II during the Wound Healing Response of Fibroblasts

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Abstract. Myosin I is present in Swiss 3T3 fibroblasts and its localization reflects a possible involvement in the extension and/or retraction of protrusions at the leading edge of locomoting cells and the transport of vesicles, but not in the contraction of stress fibers or transverse fibers. An affinity-purified polyclonal antibody to brush border myosin I colocalizes with a polypeptide of 120 kD in fibroblast extracts. Within initial protrusions of polarized, migrating fibroblasts, myosin I exhibits a punctate distribution, whereas actin is diffuse and myosin II is absent. Myosin I also exists in linear arrays parallel to the direction of migration in filopodia and microspikes, established protrusions, and within the leading lamellae of migrating cells. Myosin II and actin colocalize along transverse fibers in the lamellae of migrating cells, while myosin I displays no definitive organization along these fibers. During contractions of actin-based fibers, myosin II is concentrated in the center of the cell, while the distribution of myosin I does not change. Thus, myosin I is found at the correct location and time to be involved in the extension and/or retraction of protrusions and the transport of vesicles. Myosin II-based contractions in more posterior cellular regions could generate forces to separate cells, maintain a polarized cell shape, maintain the direction of locomotion, maximize the rate of locomotion, and/or aid in the delivery of cytoskeletal/contractile subunits to the leading edge.

In recent years, the absolute role of myosin II in nonmuscle cell locomotion has been questioned. Immunofluorescence localization of myosin II in fixed cells (Conrad et al., 1989) and microinjection of fluorescent analogs of myosin II into living cells (DeBiasio et al., 1988) show that myosin II is absent from initial protrusions at the leading edge of polarized, migrating Swiss 3T3 fibroblasts. In addition, microinjection of antibodies against myosin II into fibroblasts (Höner et al., 1988) and Acanthamoeba (Sinard and Pollard, 1989) does not stop either protrusive events or cellular translocation, although the morphology of the fibroblasts is dramatically altered. Amoeboid cells lacking functional myosin II (De Lozan and Spudich, 1987; Knecht and Loomis, 1987; Manstein et al., 1989) retain motile phenotypes, although the direction and speed of migration are not equivalent to those observed in wild-type cells (Wessels et al., 1988). It is the present consensus that cell locomotion is impaired when myosin II is absent, but not blocked. In contrast, the contraction of fibers such as stress fibers (Giuliano and Taylor, 1990; Kolega et al., 1991), transverse fibers in migrating fibroblasts during wound healing (DeBiasio et al., 1988), and the cleavage furrow of dividing cells (Pollard et al., 1990; Cao and Wang, 1990; Fukui and Inoué, 1991; De Lozan and Spudich, 1987) appears to involve myosin II.

Some motile activities that appear to occur independently of myosin II may be dependent upon myosin I. Myosin I is a lower molecular weight, nonfilamentous form of myosin that has been studied extensively in Acanthamoeba, Dictyostelium, and intestinal brush border cells (for reviews see Korn and Hammer, 1990; Pollard et al., 1991). Based on its intracellular location and in vitro regulation, myosin I could be the motor for several types of cellular movements. It has been immunolocalized at the anterior of migrating Dictyostelium cells (Fukui et al., 1989), associates directly with membranes, phospholipid micelles and vesicles (Adams and Pollard, 1989; Gadesi and Korn, 1980; Hammer et al., 1984; Hayden et al., 1990; Miyata et al., 1989), and transports membranes in vitro (Adams and Pollard, 1986; Moosiker et al., 1989). It cross-links actin filaments into a gel and, in the presence of ATP, causes gel contraction (Fujisaki et al., 1985). These properties of myosin I suggest a possible role in leading edge extension and/or retraction, membrane ruffling, and vesicle and organelle transport.
Isosomes of myosin I have been found in *Acanthamoeba* (Jung et al., 1987; Jung et al., 1989b), *Dicyostelium* (Jung et al., 1989a; Jung and Hammer, 1990; Titus et al., 1989) and intestinal brush border (Halsall and Hammer, 1990). Recently, myosin I isosomes were identified in several mammalian tissues (Barylko et al., 1991; Coluccio, 1991; Wagner et al., 1992) and found to be more closely related in structure and activity to myosin I from brush border than amoeboid cells (Barylko et al., 1991).

We wanted to determine whether the localization of myosins I and II in higher eukaryotic cells was consistent with an involvement in motile activities including extension and/or retraction of protrusions, vesicle transport, contraction of actin-based fibers, and the locomotion of fibroblasts during wound healing. We describe here for the first time the immunolocalization of the motor proteins, myosins I and II, in migrating mammalian cells and cells in which stress fibers are actively contracting.

**Material and Methods**

**Cell Culture**

Swiss 3T3 fibroblasts (CCL92, American Type Culture Collection, Rockville, MD, passage 119-128) were cultured in DME (Gibco Laboratories, Grand Island, NY) pH 7.4 containing 2 mg/ml penicillin, 0.05 mg/ml streptomycin, and 10% calf serum in a humidified, 5% CO2 atmosphere. Culture of nonconfluent monolayers of cells and serum-deprivation were according to Giuliani and Taylor (1990). Actin-based fibers in the serum-deprived cells were induced to contract by the addition of DME containing 10% calf serum (Giuliano and Taylor, 1990).

Cell culture and in vitro wounding of confluent monolayers were identical to those described previously (Conrad et al., 1989). The major stages of wound healing we have studied, from the initial wounding of a confluent monolayer to the first cell division, are as follows (average hours post-wounding are indicated in parentheses): (a) extension and retraction of protrusions (immediate to 24); (b) formation of filopodia (immediate to 24); (c) formation, transport, and contraction of transverse fibers (immediate to 8-10); (d) polarization of cells (immediate to 3); (e) polarized migration (1-24); (f) early phase of lamellar contraction (1-4); (g) later phase of lamellar contraction (4-10); (h) separation of neighboring cells (4-10); (i) highly motile stage of separated cells without well defined transverse fibers (8-24); and (j) cell division (24).

**Preparation of Cell Extracts, Gel Electrophoresis, and Immunoblotting**

Swiss 3T3 fibroblasts were plated at a density of 2 × 10^5 cells per 100-mm dish. 48 h later, cells were rinsed three times with PBS, scraped with a rubber policeman after adding 500 μl of boiling 5X sample buffer (312 mM Tris, pH 6.8, 10% (wt/vol) SDS, and 25% 2-mercaptoethanol), and immediately boiled for 2 min. The cell homogenate was subjected to SDS-PAGE with 4-16% acrylamide gradient run at 8 W for 4 h. Prestained molecular weight markers (Bio Rad Laboratories, Richmond, CA) were used for determination of immunoreactive polypeptide molecular weights. After electrophoresis, the gel was washed with three 10-min changes of 10 mM CAPS, pH 11 and the separated proteins were electroelutically transferred to a charged nylon membrane (Zeta-probe, Bio Rad Laboratories) for 16 h at 25 V, 0°C in the same buffer. After transfer, the membrane was placed in blocking buffer (6% bovine casein, 1% polyvinylpyrrolidone-40, 3 mM NaNO3, 0.1 mM EDTA, in PBS, pH 7.0) for 4 h then incubated overnight with an affinity-purified polyclonal antibody to chicken epithelial brush border myosin I (Collins et al., 1990) diluted 1:10,000 in blocking buffer. After three 5-min washes in 0.3%Tween in PBS, pH 7.4, the membrane was incubated for 2 h in goat anti-rabbit IgG-conjugated alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL) diluted 1:1,000 in blocking buffer. The membrane was then washed with five 5-min changes of 0.3% Tween in PBS, two 5-min changes of assay buffer (50 mM Tris and 1 mM MgCl2, pH 9.5), and incubated for 5 min in assay buffer containing 400 μM 3-(4-methoxyphenyl)1,2-dioxetane-3,2' tricyclo-

**Immunofluorescence**

Cells were fixed for 10 min in 2% formaldehyde in cytoskeletal stabilizing (CS) buffer (137 mM NaCl, 5 mM KCl, 1.1 mM NaHPO4, 0.4 mM KH2PO4, 4 mM NaHCO3, 2 mM MgCl2, 5.5 mM glucose, 2 mM EGTA, 5 mM PIPES, pH 6.1, 37°C). After three 5-min rinses in CS buffer, the cells were permeabilized by incubation in CS buffer containing 0.5% Triton X-100 for 40 s. After rinsing (3×, 5-rain each), the cells were incubated for 60 min at room temperature in 40 μl of a primary antibody mixture containing Bodipy-phallacidin (Molecular Probes, Inc., Eugene, OR) to visualize actin, an affinity-purified polyclonal antibody to chicken epithelial brush border myosin I (Collins et al., 1990), and a monoclonal antibody to myosin II light chains (Sigma Chemical Co., St. Louis, MO; recognizes myosin II in fibroblast extracts as a single band on Western blots, data not shown) prepared in CS buffer plus 10 mg/ml BSA. The cells were rinsed as before, and transferred to 40 μl of a secondary antibody mixture containing rhodamine (TRITC)-conjugated affinity-purified goat anti-rabbit IgG and Cy-5-conjugated affinity-purified sheep anti-mouse IgG (Biological Detection System, Inc., Pittsburgh, PA) for an additional 60 min. After three additional washes in CS buffer, coverslips were mounted in Gelvatol (Monsanto Chem. Co., Springfield, MA) containing 30% glycerol with 1% n-propyl gallate added as an antibleaching agent. In control experiments where the primary antibodies were omitted, the secondary antibodies were omitted, or the cells were treated with the appropriate preimmune serum, no labeling of cells was observed.

1. Abbreviation used in this paper: CS, cytoskeletal stabilizing.

![Figure 1](https://example.com/figure1.png)
Figure 2. Triple immunofluorescent labeling of polarized, migrating Swiss 3T3 fibroblasts during wound healing for actin (A, D), myosin II (B, E), and myosin I (C, F). Actin and myosin I are found within initial protrusions (A, C, arrows) and filopodia (D, F, arrowheads), while myosin II is concentrated at the base of initial protrusions, other transverse fibers throughout the lamellum, and absent from initial protrusions (B) and filopodia (E). Arrow in upper left corner indicates direction of cellular migration. See Fig. 8, A and B, for color overlays of these images. Bar, 10 μm.

**Fluorescent Analog of Myosin II**

The acetamido-tetramethylrhodamine analog of smooth muscle myosin II (AR-myosin-II) was prepared as described by DeBiasio et al. (1988). The concentration of AR-myosin-II used for microinjection was between 3 and 6 mg/ml and ~5% of the cell’s volume was injected. Cells microinjected with AR-myosin-II were allowed to recover for 1-1.5 h before an experiment. We maintained cells in a physiological environment on the microscope stage in a sealed chamber of our own design (Giuliano et al., 1989).

**Image Processing**

Multi-mode microscopy was identical to Conrad et al. (1989). Sets of fluorescent images were acquired sequentially for each fluorophore. Companion images were enhanced in an identical manner using image processing techniques including unsharp masking (Conrad et al., 1989). For the composite, overlay images, the companion images were displayed as a separate color with myosin I displayed in the red channel and myosin II in the green channel. A resultant yellow color represents those areas where the...
red and green images overlap. The final images selected are representative of the results observed during the various stages of wound healing-induced cellular migration or serum-deprivation/-stimulation. Greater than 300 cells were studied for each condition and the images were chosen to reflect the average patterns of immunofluorescence.

**Results**

**A Polyclonal Antibody Specific for Chicken Brush Border Myosin I Recognizes a Polypeptide of 120 kD in Swiss 3T3 Fibroblasts**

We probed the SDS-soluble fraction of a Swiss 3T3 cell extract with a polyclonal antibody specific for chicken brush border myosin I (Collins et al., 1990). This antibody does not cross-react with myosin II and it inhibits myosin I-based motility in the sliding filament in vitro motility assay (Collins and Matsudaira, 1991). The antibody recognized one polypeptide at 120 kD (Fig. 1). Lower molecular weight bands, including one at 70 kD, appeared if the cell extracts were not electrophoresed immediately after preparation. Because the antibody recognizes a similar epitope in purified brush border myosin I, and because the molecular weight of the antigen in fibroblasts is similar to that of other vertebrate myosin I proteins (Ezzell et al., 1992; Wagner et al., 1992), we believe that it represents Swiss 3T3 myosin I.

**Myosin I Exists in Initial Protrusions and in Newly Formed Filopodia, While Myosin II Exhibits a Delayed Penetration into These Structures**

Fig. 2 shows the distribution of actin, myosin II, and myosin I within initial protrusions (Fisher et al., 1988) and filopodia of migrating cells at an early stage in the wound healing response. The maintenance of filopodial and lamellipodial structure required careful attention to the details of fixation, buffers, and procedures (Conrad et al., 1989). At the time of fixation, the initial protrusion in Fig. 2, A–C, lacked detectable substructure with video-enhanced contract microscopy, although by the time fixation was complete, actin microspikes had begun to form (Fig. 2 A). Myosin I appeared as discrete punctate spots throughout initial protrusions (Fig. 2 C, arrows), while myosin II (Fig. 2 B) was absent from initial protrusions, concentrated at the base of protrusions as well as in more distal transverse fibers, and associated with the membrane at the edge of the protrusions. Initial protrusions containing actin and myosin I, but devoid of myosin II, were also observed at the base of newly formed filopodia (Fig. 2, D–F; arrow). Actin (Fig. 2 D, arrowhead) and myosin I (Fig. 2 F, arrowhead) colocalized along the length of the filopodia, while myosin II (Fig. 2 E) was excluded. Myosin II penetrated into filopodia and protrusions in a time-dependent manner as described previously (Conrad et al., 1989; DeBiasio et al., 1988). Color overlays (Fig. 8, A and B) of the companion myosin I and myosin II images from Fig. 2 highlight the asymmetric distribution of myosin I in filopodia and initial protrusions. In contrast, established protrusions (Fisher et al., 1988; Conrad et al., 1989; DeBiasio et al., 1988; Fig. 3, A–C, arrows) contained linear arrays of actin, myosin I, and myosin II that often colocalized, as well as a punctate distribution of myosin I throughout the established protrusions (Fig. 3 C).
Myosin II, But Not Myosin I, Is Involved in the Arrays in the Leading Lamellae of Migrating Cells, While Myosin II Exhibits a Periodic, Semisarcomeric Organization within Transverse Fibers

Within the leading lamellae of cells exhibiting either initial (Fig. 2) or established (Fig. 3) protrusions, actin was distributed continuously along transverse fibers (Figs. 2 A and 3 A), while myosin II was found in its typical periodic, semisarcomeric distribution along the length of the transverse fibers (Figs. 2 B and 3 B). Similar to its distribution within lamellipodia, myosin I appeared as discrete punctate spots distributed diffusely throughout the leading lamella (Figs. 2 C and 3 C). Short, linear arrays of myosin I were also found initially in the perinuclear region of polarized migrating cells sometimes extending well into the lamellum, parallel to the direction of migration (Fig. 3 C, arrowheads).

Myosin II, But Not Myosin I, Is Involved in the Contraction of Transverse Fibers during the Lamellar Contractile Phase of Wound Healing

During wound healing-induced cellular migration, contractions of transverse fibers in the leading lamellae allow cells to pull away from neighbors and restructure cell shape for locomotion (DeBiasio et al., 1988; Hahn et al., 1992). We examined the dynamic nature of the transverse fibers as they formed, transported, and contracted using time-lapse fluorescence imaging of a myosin II fluorescent analog. Fig. 4 shows a series of sequential images of a living Swiss 3T3 fibroblast along a wound edge that was microinjected with AR-myosin II. Transverse fibers formed at the base of protrusions and were transported centripetally toward the nucleus (compare the location of the fiber indicated by the black arrowhead in the sequential images). The myosin II pattern was semisarcomeric as soon as the transverse fibers could be detected at the base of protrusions. The transverse fibers began to contract soon after they formed (compare the location of the arrow in sequential images) coincident with their translocation toward the perinuclear region in the rear of the cell. These time-lapse images show that cells in the lamellar contractile phase of wound healing exhibit polarized fiber formation, transport, and contraction involving myosin II.

Fig. 5 (A–C) shows a fibroblast in an early stage of lamellar contraction involving the transverse fibers. As discussed above, myosin II (Fig. 5 B) appeared in a semisarcomeric distribution along the length of the actin-containing transverse fibers, while myosin I (Fig. 5 C) was found as diffuse, punctate spots and linear arrays of punctate spots throughout the cell. Fig. 5 (D–F) shows a cell in the later stages of lamellar contraction that separate neighboring cells during the wound healing process. The semisarcomeric distribution of myosin II along the transverse fibers (Fig. 5 B), observed in earlier stages of the lamellar contraction, was not apparent in the later stages (Fig. 5 E) of lamellar contraction, since the fibers had shortened maximally (see also Giuliano and Taylor, 1990; Kolega et al., 1991). Also, very little myosin II was found near the edges of the cell, most likely due to contraction of the fibers resulting in the retraction of myosin II from the periphery (Fig. 5 E). Myosin I (Fig. 5 F), on the other hand, was found in diffuse, punctate spots, as well as linear arrays in the entire cell, including the peripheral regions. There was no apparent alteration in the myosin I distribution before or after periods of lamellar contraction. Similar to cells in earlier stages of wound healing (Fig. 3), cells undergoing lamellar contractions of transverse fibers exhibited a subset of myosin I in linear arrays extending from the perinuclear region toward the leading edge parallel to the direction of migration (Fig. 5 F, arrows).

Myosin I Exhibits a Slight Bias to the Front, While Myosin II Is Reduced at the Front of Motile Cells

Myosin I (Fig. 6 B) was found throughout migrating cells with a slight bias at the leading edge, especially in filopodia. Myosin II (Fig. 6 A) was reduced relative to myosin I at the leading edge and concentrated in the cortical and posterior regions. Myosin II appeared to lose fiber structure in the...
Figure 5. Triple immunofluorescent labeling of polarized, migrating Swiss 3T3 fibroblasts during early (A-C) and later (D-F) stages of lamellar contractile phase of wound healing showing the distribution of actin (A, D), myosin II (B, E), and myosin I (C, F). During the early stage of lamellar contraction, usually from 1-4 h postwounding, myosin II (B) is found in a semisarcomeric distribution along the actin-containing transverse fibers. After extensive lamellar contraction (E), usually between 4 and 6 h postwounding, the semisarcomeric distribution is not evident, presumably because fiber contraction has condensed the precontraction pattern. The cellular distribution of myosin I is similar in early (C) and late (F) lamellar contraction of the transverse fibers. A subset of myosin I (F, arrows), in a linear pattern, extends from the perinuclear region towards the leading edge, parallel to the direction of cellular migration. White arrow indicates direction of cellular migration for all images. Bar, 10 μm.
perinuclear region in the rear of the cell. A color overlay (Fig. 8 C) highlights this differential distribution of myosins I and II in a highly motile cell that had separated from neighboring cells. Note that myosin I was present in microspikes, protrusions, and filopodia at the leading edge, but myosin II was not. In addition, transverse fibers became less demarcated in the migrating cells following separation from neighboring cells.

**Contraction of Stress Fibers Induces a Concentration of Myosin II, But Not Myosin I**

Contractions of stress fibers containing actin and myosin II were also visualized in nonmotile, serum-deprived cells upon stimulation with complete serum (Giuliano and Taylor, 1990). When fibroblasts are deprived of serum they flatten, become quiescent and nonmotile, and develop extensive arrays of stress fibers. Addition of complete serum, purified growth factors, or cytochalasin induces the contraction and ultimate disappearance of many stress fibers (Giuliano and Taylor, 1990; Kolega et al., 1991). Thus, the serum-deprived fibroblast is a valuable nonmuscle model system in which to investigate the regulation and dynamics of myosin II-based contractions. Fig. 7 shows the effect of serum-stimulation on the distribution of myosins I and II. Myosin II (Fig. 7 A) was localized along contracting fibers, while myosin I (Fig. 7 B) appeared in a diffuse, punctate distribution throughout the cell and was not significantly concentrated in contracted regions.

**Discussion**

**Myosin I as a Motor Molecule in the Lamellum and Lamellipodia of Migrating Cells**

Fibroblast locomotion during wound healing is the result of a series of coordinated cellular events, including lamellipodial extension and retraction, cortical transport of actin and myosin II, contraction of transverse fibers, and tail retractions. The presence of myosin I in protrusive structures of migrating fibroblasts suggests that this motor could be part of the motive force underlying lamellipodial extension and/or retraction. Because the myosin I organization in lamellae does not change significantly before or after periods of lamellar contraction, it is unlikely that myosin I plays a direct role in the contraction of transverse fibers. A subset of myosin I is found extending from the perinuclear region towards the leading edge parallel to the direction of cellular migration in subtle, linear arrays. Also, discrete, punctate spots of myosin I are found relatively uniformly dispersed within the lamellae and in the perinuclear region. The function of these linear arrays and diffuse spots of myosin I is unknown. However, myosin I has been immunolocalized to vesicles in brush border cells (Drenckhahn and Dermietzel, 1988) and supports vesicle traffic in vitro (Adams and Pollard, 1986). It is interesting to speculate that the punctate regions of myosin I are vesicles coated with associated myosin I, while the linear arrays represent closely spaced myosin I associated vesicles that are moving along cytoskeletal tracks towards the anterior leading edge of migrating cells. A fluorescent analog of myosin I combined with motion analysis of specifically-labeled organelles should form a good test of this possibility.

**Myosin II as a Motor Molecule Regulating Cell Shape and the Direction and Rate of Migration**

Because myosin II is not found within initial protrusions (see also Conrad et al., 1989; DeBiasio et al., 1988) and because protrusive activity still occurs in cells lacking functional myosin II (Höner et al., 1988; Sinard and Pollard, 1989; DeLozanne and Spudich, 1987; Knecht and Loomis, 1987; Manstein et al., 1989; Wessels et al., 1988), this motor can-
not be primarily responsible for force generation in cell locomotion. However, myosin II-based contractions could be involved in at least four force generating components of the locomotion of fibroblasts during wound healing. First, contractions of transverse fibers during the lamellar contractile phase of wound healing could separate cells and restructure the cell shape to optimize locomotion. Second, the cortical contraction of transverse fibers and of a less structured cortex in highly motile cells could optimize polarized migration by acting as a steering mechanism to maintain the direction of migration, through the polarized formation, transport, and contraction of the cortical actomyosin II. Third, this cortical contraction could regulate the rate of migration, maintain an optimal polarized shape, and/or aid in the delivery of cytoskeletal/contractile subunits to the leading edge via anterior edge-directed, positive hydrostatic pressure. Fourth, surface tension mediated by a contractile, cortical actomyosin II gel network could inhibit lamellipodial extension in lateral and posterior regions (Spudich, 1989). The possible force generation roles of myosins I and II could also be complemented by other forces that could play a role in pseudopod extension including actin polymerization (Tilney and Inoué, 1985), osmotic gel swelling (Oster and Perelson, 1987), and cortical expansion (Condeelis et al., 1990; Condeelis, 1992).

The Effects of Ca\textsuperscript{2+} on the Motor Activities of Myosins I and II

In fibroblasts, the distribution of myosins I and II is not exclusive, and differential regulation of the motors may be required to coordinate pseudopod extension with lateral contraction and tail retraction. One possible mechanism of regulation is Ca\textsuperscript{2+}. Under certain conditions (see Barylko et al., 1991) vertebrate myosins I and II are differentially responsive to regulation by Ca\textsuperscript{2+} (Collins et al., 1990; Collins and Matsudaira, 1991). High Ca\textsuperscript{2+} causes a loss of calmodulin light chains from the myosin I complex and results in an inhibition of motility and actin-activated ATPase activity (Collins et al., 1990; Swanljung-Collins and Collins, 1991), while myosin II is activated by elevated free Ca\textsuperscript{2+} (Kamm and Stull, 1989; Sellers and Adelstein, 1987). Elevated Ca\textsuperscript{2+} levels are observed in the lamellae where transverse fibers have been shown to contract (Hahn et al., 1992). There is also a gradient of free Ca\textsuperscript{2+}, lowest at the leading edge and maximal in the more posterior regions of locomoting cells (Taylor et al., 1980; Brundage et al., 1991; Hahn et al., 1992). The elevated Ca\textsuperscript{2+} in more posterior
regions of the cell would maximize myosin II-based contractions, while simultaneously inactivating myosin I. Conversely, a low concentration of Ca$^{2+}$ at the leading edge would promote the activity of myosin I over myosin II motors. Thus, increasing motile activity at the leading edge, via myosin I dynamics and/or other low calcium-based force generating mechanisms, coupled to actomyosin II-based contractions in regions posterior to the leading edge, would
ensure polarized migration. This differential regulation of overlapping motors would allow fibroblasts to respond to external cues and alter the position of their leading edge without an extensive redistribution of myosin motors. In addition to the high degree of spatial control of free calcium levels (Hahn et al., 1992), complete control of motor activity could involve other regulatory processes (Barylko et al., 1991) such as the phosphorylation of myosin II (Giuliano et al., 1991; Kolega et al., submitted for publication).

Furthermore, in the nonmotile, serum-deprived fibroblast, the intracellular Ca^{2+} level is known to be \(<10^{-7}\) M (McNeill et al., 1985; Tucker and Fay, 1990; Hahn et al., 1992) yet a constitutive formation, transport, and disassembly of stress fibers ensues (Giuliano and Taylor, 1990). Although a fraction of the myosin II in these cells is phosphorylated and therefore generating cytoplasmic tension (Giuliano et al., 1992), stress fiber shortening does not occur suggesting that another force generating molecule, such as myosin I, might be responsible for some of the cytoskeletal dynamics we observe. High resolution cellular mapping of free calcium and myosin motors is currently under investigation.

**Myosins I and II as Mediators of Cortical Flow**

Fibroblasts undergoing the wound healing response exhibit a polarized, cortical flow or cycle of formation, transport, contraction and disassembly of an actomyosin II-based fiber system starting at the leading edge and ending in the posterior, perinuclear regions (DeBiasio et al., 1988; Fisher et al., 1988; this study). This cortical flow is similar to that identified in free living amoeba (Taylor and Fechheimer, 1982; Bray and White, 1988). The transverse fibers form and begin transporting in the low calcium, anterior region of the cell. Therefore, the initial transport process must involve a low calcium mechanism. Myosin I might play a role in addition to actin assembly. However, contraction of transverse fibers appears to be maximal as the transported fibers approach the perinuclear region in the rear of locomoting cells where they disappear. The gradient of free Ca^{2+} has been identified in locomoting fibroblasts with the minimum at the leading edge and the maximum in more posterior regions (Hahn et al., 1992) which is similar to the gradient detected in free living amoeba (Taylor et al., 1980) and in eosinophils (Brundage et al., 1991). This gradient would support the hypothesis that the actomyosin II-based transverse fibers function in part as a contractile “corset” resulting in some or all of the functions described above. The solution-contraction coupling hypothesis can account for the dynamic assembly, contraction, disassembly, and recycling of this cytoskeletal/contractile system (Janson et al., 1991; Kolega et al., 1991; Hahn et al., 1992). Although organized differently in different cells, the actomyosin II cytoskeletal/contractile system may exhibit some similar dynamics and functions in cell movements (Taylor and Condeelis, 1979).

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