Myosin II dynamics and cortical flow during contractile ring formation in *Dictyostelium* cells

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Myosin II is a major component of a contractile ring. To examine if myosin II turns over in contractile rings, fluorescence of GFP–myosin II expressed in *Dictyostelium* cells was bleached locally by laser illumination, and the recovery was monitored. The fluorescence recovered with a half time of 7.01 ± 2.62 s. This recovery was not caused by lateral movement of myosin II from the nonbleached area, but by an exchange with endoplasmic myosin II. Similar experiments were performed in cells expressing GFP–3ALA myosin II, of which three phosphorylatable threonine residues were replaced with alanine residues. In this case, recovery was not detected within a comparable time range. These results indicate that myosin II in the contractile ring performs dynamic turnover via its heavy chain phosphorylation. Because GFP–3ALA myosin II did not show the recovery, it served as a useful marker of myosin II movement, which enabled us to demonstrate cortical flow of myosin II toward the equator for the first time. Thus, cortical flow accompanies the dynamic exchange of myosin II during the formation of contractile rings.

**Introduction**

During cell division, animal cells and lower eukaryotic cells such as *Dictyostelium* construct contractile rings, which are mainly composed of actin and myosin II filaments. In addition to the classic experiment in which microinjection of antityrosin antibodies inhibited cell division (Mabuchi and Okuno, 1977), recent targeting of the myosin II gene established that myosin II plays an essential role in the contraction of contractile rings (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Kitayama et al., 1997). It is now generally accepted that the force exerted by the interaction between these filaments is believed to constrict the contractile rings and divide a cell into two daughter cells (Wolf et al., 1999; Sanger and Sanger, 2000).

FRAP and fluorescence loss in photobleaching (FLIP)* methods have been applied in order to observe and measure the diffusion activity of membrane molecules, and actin dynamics during the pseudopods extension of migrating cells (Jacobson et al., 1977; Wang, 1985). In this study, we combined these techniques with the recent recombinant green fluorescent protein (GFP)-tagged protein technology (White and Stelzer, 1999), to examine if myosin II is simply transported in a static manner or has any dynamic aspects during the construction of contractile rings.

The assembly of *Dictyostelium* myosin II into filaments is regulated by phosphorylation of its heavy chains. The phosphorylation of three threonine residues in its tail domain by specific kinase induces the disassembly of filaments (Kuczmarski and Spudich, 1980; Egelfhoff et al., 1991). The expression of a mutant myosin II heavy chain (MHC) gene (3ALA), having converted these three threonine residues to alanine residues in MHC null cells enables one to assess the role in vivo of the phosphorylation regulation of these threonine residues. The cell division and development of fruiting bodies of these mutant cells are impaired, however the defects are not severe. On the other hand, in the case of 3ASP, in which three aspartate residues replace the three threonine residues in order to mimic the phosphorylated state, the transformed cells can neither divide in suspension nor perform the development normally (Egelfhoff et al., 1993). The phosphorylation of heavy chains influences intracellular localization of myosin II as well. 3ALA myosin II localizes only in the cortex (Yumura and Uyeda, 1997a), whereas 3ASP myosin II is diffusely distributed throughout the endoplasm and does not localize to specific cortical regions such as contractile rings (Sabry et al., 1997). Thus, the myosin II’s cortical localization is crucial for cell division and development, although the regulation of heavy chain phosphorylation is not essential for the viability of cells.
It is possible to view contractile rings as a rigid and more or less stable structure, partly because it contains actin-bundling proteins such as α-actinin (Fujisawa et al., 1978), although it temporally appears and disappears during the cell division process. This study brings a different view by showing that myosin II turns over rapidly and continuously. In addition, it demonstrates the cortical flow of myosin II toward the equator, as detected for the first time in Dictostelium cells. Together, it is concluded that both the cortical flow and the dynamic exchange of myosin II occur simultaneously during the formation of the contractile ring.

**Results**

**Rapid turnover of myosin II at the contractile ring**

GFP-tagged myosin II was expressed extrachromosomally in MHC null cells as described (Moores et al., 1996). This means that all MHCs in the cells were tagged with GFP. To assess if myosin II turns over in the contractile rings by the FRAP method, a small area of a contractile ring of cells expressing GFP-tagged myosin II was photobleached by scanning laser illumination (Fig. 1). The fluorescence intensity of the bleached region shows rapid recovery after photobleaching (Fig. 1 B). A half time of recovery \( t_{1/2} \) was 7.01 ± 2.62 (SD; \( n = 16 \)) s. The time course of recovery showed a simple exponential rise to a maximum by curve fitting (Fig. 1, B and C), indicating that this process is mainly composed of a single enzymatic reaction.

GFP is reported to undergo repeated cycles of fluorescent emission, blinking (Dickson et al., 1997). However, the possibility that the observed FRAP may be due to this property of GFP was eliminated by the observation that there was no recovery when the fluorescence of the whole cell area was bleached (data not shown).

The application of laser illumination for photobleaching was limited to the cell cortex. If it was applied to the inner region (the endoplasm), the curve of recovery process showed different kinetics, reflecting diffusion of myosin in the endoplasm (unpublished data). In the present FRAP experiments, only the behavior of cortical myosin II was analyzed.

**Recovered myosin II came from the endoplasm**

To examine from where the myosin II recovered in the bleached region came, two rectangular regions parallel to the cleavage axis were bleached, as shown in Fig. 2. If the fluorescence intensity of the middle region between these two bleached regions decreases in accordance with the fluorescence recovery in the bleached regions, then it will suggest that myosin II moves along the cortex rapidly. Otherwise, the recovered myosin II must be derived from the endoplasm to be exchanged with the existing myosin II. The fluorescence intensity of the middle region did not show any decrease (Fig. 2 B). Furthermore, the fluorescence intensity of any region within the bleached areas increased at the same rate. If the fluorescence intensity of the limb region of bleached areas increased faster than the inner region, it could indicate that myosin II slid along the cortex. Therefore, the recovered myosin II did not slide from a neighbor cortex but came from the endoplasm. The fluorescence recovery was observed even after photobleaching of a whole contractile ring (Fig. 3), further supporting this possibility.

To examine if the cortical myosin II in interphase cells behaves in a similar way to that in contractile rings, a small area along the cortex of an interphase cell was photobleached (Fig. 4 A). The time course of recovery was quite similar to that of contractile rings. To assess where the exchanged myosin II was translocated to by the FLIP method, the fluorescence of all region except two small cortical areas was bleached (Fig. 4 C). The fluorescence of nonbleached areas decreased in a reverse manner of recovery and was diffused in the endoplasm. Note that immediately after photobleaching, the fluorescence intensity increased in the endoplasm. These observations suggest that cortical myosin II is rapidly exchanging with endoplasmic myosin II. In addition, because there were not any changes in the distance between the two areas, it is suggested that most of myosin II does not move laterally in the cortex within a short time.

**Phosphorylation of heavy chains is required for the turnover**

It was suggested that the localization of myosin II to the cortex requires the dephosphorylation of heavy chains because 3ASP mutant myosin II, carrying aspartate residues in place of phosphorylatable threonine residues, does not localize to the cortex but disperses in the endoplasm (Egelhoff et al., 1993). Conversely, 3ALA mutant myosin II localizes only to the cortex (Yumura and Uyeda, 1997a). Confocal microscopy of cells expressing the GFP-version of this mutant myosin confirmed its cortical localization by serial optical sectioning (Fig. 5).
FRAP experiments were performed in the cleavage furrow of cells expressing GFP–3ALA myosin II (Fig. 6). Interestingly, fluorescence of the bleached region did not recover. In a complementary FLIP assay, the fluorescence of all areas except two small cortical areas was bleached in interphase GFP–3ALA myosin II cells (Fig. 7). The fluorescence of nonbleached areas neither decreased nor diffused into the endoplasm. As observed in wild-type cells, there were not any changes in the distance between the two areas. These observations strongly suggest that the heavy chain phosphorylation is responsible for the turnover of cortical myosin II.

**Phosphorylation accompanies the myosin II translocation to the endoplasm**

To gain further support for the hypothesis that the translocation of myosin II from the cortex to the endoplasm requires heavy chain phosphorylation, Triton X-100 cytoskeleton assays were performed. Triton X-100–insoluble cytoskeletons (Triton ghosts) contract upon the addition of 1 mM ATP (Yumura, 1991). At this time, almost all of myosin II associated with the cytoskeletons was released into the supernatant fraction (Fig. 8, ○). In accordance with the release, MHCs were phosphorylated (Fig. 8, bars). On the contrary, most of 3ALA myosin II was not released (Fig. 8, ●), although Triton ghosts of 3ALA cells could contract upon the addition of ATP. Similar experiments were performed by Egelhoff et al. (1993), in which ATP was added to the cells together with Triton X-100. In this case, it was unclear which fraction, the supernatant or Triton ghosts, contained the kinase activity. The present experiments indicate that the heavy chain kinase responsible for the release of myosin II was associated with Triton ghosts. Since the Triton ghosts are mainly composed of cortical cytoskeletons, the release of myosin II from the Triton ghosts represents its translocation toward the endoplasm (Yumura, 1991). Similar results were obtained from the previous experiments using membrane–cytoskeleton complexes, in which myosin II is released from them by the treatment with ATPγS as substrate for phosphorylation, followed by the addition of ITP as an energy source for myosin motor activity (Yumura and Kitanishi-Yumura, 1992, 1993). Thus, these in vitro experiments also support the model that the translocation of myosin II from the cortex to the endoplasm requires the phosphorylation of heavy chains.

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**Figure 2.** Myosin II does not move laterally in the contractile ring. Two rectangle areas were photobleached (0:00), and a fluorescence recovery process was followed. The fluorescence (arrow 1) between the photobleached areas did not decrease (plot 1 in B). On the other hand, the fluorescence in the bleached area (arrow 2 in A, plot 2 in B) recovered with a kinetic curve similar to that shown in Fig. 1 B. A half time of recovery was 7.48 ± 2.43 (n = 7).

**Figure 3.** Photobleaching of a whole contractile ring. The fluorescence of a whole contractile ring region was bleached, but recovery was still observed. Note that the fluorescence intensity in the area except the contractile ring decreased after photobleaching. The number at the left corner of each image indicates time after photobleaching (in seconds). This is a representative figure of observed eight different cells. Bar, 5 μm.
Cortical myosin II filaments move toward the equator

The fluorescence recovery of GFP-WT myosin II cells was too rapid to trace the bleached region even if myosin II filaments moved along the cortex. The property of nonrecovery of GFP–3ALA myosin II cells after photobleaching made it possible to mark the cortical myosin II and to observe it for a longer period. When parts of a cleavage region in early telophase were bleached, the unbleached cortical fluorescence flanking the furrow moved toward the equator (Fig. 9). Because 3ALA myosin II was in filamentous form in the cortex as observed in Fig. 5, it is concluded that myosin II filaments move toward the equator along the cortex. This is the first direct evidence that cortical myosin II flows toward the equator in Dictyostelium. The rate of the flow of individual filaments might not be uniform because slight increase in fluorescence in the central part of the bleached region occurred preceding the flow of major fluorescence toward the equator. However, they still moved toward the equator as a mass.

In conclusion, the cortical myosin II filaments in wild-type cells probably go through a rapid exchange with endoplasmic myosin II and move toward the equator along the cortex simultaneously.

Discussion

In this study, we have demonstrated that myosin II in the contractile ring exchanges rapidly and continuously with the endoplasmic myosin II. This exchange is mediated by the phosphorylation of the heavy chains. In addition, cortical myosin II flows along the cortex toward the equator. This and previous studies (Yumura and Fukui, 1985; Yumura and Kitanishi-Yumura, 1990) suggest that the observed fluorescence of myosin II in the contractile ring and the cortex is derived from filamentous form of these molecules. Therefore, myosin II molecules must be exchanging between subunits of myosin filaments in the equatorial cortex and the pool in the endoplasm.

Subunit exchange of myosin II filaments has been examined only in muscle cells. A 50% exchange takes 2 min, 10 min, or 28 h, depending upon the in vitro systems (Trybus and Lowey, 1987; Saad et al., 1986; Davis, 1993). The exchange occurs in a matter of days in cardiac muscle (Wenderoth and Eisenberg, 1987). It should be noted that the exchange of myosin II in the cortex observed in this study is
not a simple subunit exchange, because it requires MHC phosphorylation, which induces filament disassembly, and therefore probably involves disassembly and assembly of filaments as described later.

Previous mutant experiments have revealed the important contribution of the heavy chain phosphorylation to cytokinesis. 3ALA mutant cells show partially impaired cytokinesis and exhibit slower growth in suspension (Egelhoff et al., 1993). This phenotype is intermediate between wild-type cells and MHC null cells. Wild-type cells divide and grow at normal rates both in suspension and on substrates, whereas MHC null cells become multinucleated and eventually lyse in suspension due to the inability to divide, even though they divide and grow at normal rates on substrates (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). In contrast, 3ASP mutation, having aspartate residues in place of the phosphorylatable threonine residues, mimics the phosphorylated state of the heavy chains (Egelhoff et al., 1993). This mutant myosin II did not show specific localization but diffuse distribution throughout a cell (Sabry et al., 1997). Its phenotype is similar to MHC null mutant in that it is unable to divide in a suspension culture. The dephosphorylated state of heavy chains, or the filamentous form, is thus necessary for specific intracellular localization of myosin II.

Dictyostelium has at least three MHC kinases (MHCKs): MHCK-PKC–resembling protein kinase C (Ravid and Spudich, 1989), MHCK A (Côté and Bukiejko, 1987; Kolman et al., 1996), and MHCK B (Clancy et al., 1997). MHCK-PKC might not be involved in cytokinesis because it is expressed only in the development phase of this organism. On the other hand, MHCK A is expressed also in the vegetative stage, although less than during the developmental stage. There are not any available data regarding the expression pattern of MHCK B.

It was suggested from the results of Triton ghost experiments that MHCK activities involved in the rapid exchange of myosin II could be present in the cortex. Our recent immunofluorescence study using antibodies against MHCK A shows that some fraction of MHCK A localizes to the cortex (Steimle et al., 2001). Mutants overexpressing MHCK A showed impaired cell division and development. Inversely, MHCK A knock-out mutants exhibited phenotypes similar to 3ALA mutants, suggesting that MHCK A is the major kinase in the vegetative phase of Dictyostelium cells (Kolman et al., 1996). Therefore, we speculate that MHCK A is primarily responsible for the exchange of myosin II at the contractile rings.

This study further provides direct evidence for cortical flow of myosin II toward the equator in Dictyostelium cells. DeBiasio et al. (1988, 1996) presented clear images of fibers containing myosin II moving toward the equator by fluorescence microscopy. They also observed an increase in the mean length of the fibers during late anaphase, telophase, and early cytokinesis, and a decrease during late cytokinesis. Electron microscopic observation demonstrated that these fibers are composed of laterally associated filaments of myosin II (Verkhovsky et al., 1995). In Dictyostelium cells, the dimension of observed myosin filaments showed uniform, ~0.5 μm in length, similar to that of synthetic filaments in vitro, and there has not been any evidence of the association among filaments (Yumura and Fukui, 1985; Yumura and Kitanishi-Yumura, 1990). Regarding actin, Cao and Wang (1990) showed the cortical flow of actin in dividing cultured rat kidney cells with an elegant experiment, in which microinjected fluorescent phalloidin-stabilized actin filaments moved toward the equator. When a trace amount of fluores-
cent phalloidin was introduced into the Dictyostelium cells by electroporation, the cortical actin showed a flow toward the equator of a dividing cell and toward the tail of a migrating cell (Lee et al., 1998; Fukui et al., 1999).

How does myosin II flow cortically toward the equator? Yumura and Uyeda (1997b) demonstrated that myosin II lacking ATPase activities is still collected at the equator. More interestingly, myosin II lacking the entire head region, where the ATPase activity and the actin-binding domain reside, still gathers at the equator (Zang and Spudich, 1998). Similar results were obtained in fission yeast (Naqvi et al., 1999). It is therefore strongly suggested that myosin filaments are passive passengers that ride on the flowing actin cortex. A recent, complementary study using a series of deletion and chimeric substitution mutations showed that there is no specific binding site to the cell cortex including actin on the tail domain of a Dictyostelium myosin II molecule (Shu et al., 1999). What then anchors myosin filaments to the cortex, which seems to be necessary for myosin II to move along the cortex passively? One possibility is that there might be an unknown factor that recognizes the three-dimensional structure of myosin filaments and binds them to cortical actin filaments (for reviews see Yumura, 1997; Uyeda and Yumura, 2000). This idea is supported by the observation that all fractions of 3ASP myosin II (monomeric form) are diffuse in endoplasm and do not localize in the cortical region (Sabry et al., 1997).

Does the observed rapid exchange of myosin II between the cortex and the endoplasm contribute to the recruitment of myosin II to the contractile ring? If either the association or release of myosin II in the exchange process is enhanced or reduced, this biased reaction should enhance or suppress the localization of myosin II to the equator. However, at present, we could not find evidence supporting this possibility in our FRAP and FLIP experiments, in which the association and the release of myosin II in the exchange can be identified, respectively; there was an almost equal rate of time course between them. Even during construction and destruction of a contractile ring, the exchange rate was almost similar (data not shown), indicating that such biased reaction is not a major mechanism for myosin II accumulation. However, we can not eliminate the possibility that a slight difference between these rates under detection may contribute to the recruitment of myosin II to the contractile ring. The major role of MHC phosphorylation may be to ensure the efficient recycling of myosin II molecules, so that the released molecules can be used somewhere else later on. This possibility is supported by the fact that most of 3ALA

![Figure 8. Phosphorylation-dependent release of myosin II from Triton ghosts.](image)

After 1 mM ATP was added to Triton ghosts, released myosin II from Triton ghosts was quantitated. Wild-type myosin II was readily released (○), but most of 3ALA myosin II (●) was not. The bars indicate phosphorylation of released heavy chains of wild-type myosin II. Each plot was an average of two experiments. 20% of 3ALA myosin II was released. This release was due to a partial disruption of Triton ghosts during contraction (Yumura, 1991).

![Figure 9. Cortical flow of 3ALA myosin II.](image)

(A) The equatorial region that would be constricted later was photobleached. (B) The profiles of the relative fluorescence intensity within boxed areas in the images of A were shown. The gray lines were drawn to pass each of two peaks at 0:00 and 236:22 s. The fluorescence within the boxed areas gradually moved toward the equator after photobleaching. This is a representative figure of observed 12 different cells. The movie version of this experiment can be seen at http://www.jcb.org/cgi/content/full/200011013/DC1. Bar, 5 µm.
myosin II is accumulated in a large aggregate in the cortex, as shown in Fig. 5 (this example is smaller than those in later stages), probably because it can not be released from the cortex. The time required by cleavage for 3ALA cells was much longer than that for wild-type cells (data not shown). The deficiency in recycling of myosin molecules results in retardation of cell division.

This study demonstrated that myosin II in the cortex turns over rapidly and continuously. Fig 4 C shows that cortical myosin II moves rapidly into the endoplasm. When all areas except the contractile ring were photobleached, the fluorescence intensity of the endoplasm increased, accompanying a fluorescence decrease at the contractile ring (data not shown). How is myosin II exchanged between the cortex and the endoplasm? It has been established that the phosphorylation of MHC induces disassembly of filaments in vivo and in vitro, and that monomeric myosin II does not associate with the cortex (Sabry et al., 1997). Therefore, we conclude that phosphorylation of MHC causes filament disassembly and their dissociation from the cortex in vivo. There are two explanations for the association of myosin II with the cortex. One is that monomeric myosin II is dephosphorylated and forms filaments at the cortex (Fig. 10 A). The other is that monomeric myosin II assemblies in the endoplasm, not at the cortex, and then the filaments associate with the cortex (Fig. 10 B). Evidence that filaments exist also in the endoplasm (Yumura and Fukui, 1985) and that MHC phosphatase is fractionated in the supernatant in biochemical experiments (Kuczmaszki and Pagone, 1986) support the latter explanation.

My view on myosin II dynamics during the contractile ring formation in Dictyostelium is summarized as follows. As depicted in Fig. 10 C, myosin II filaments are associated directly or indirectly with cortical actin that flows toward the equator. This motive force is independent of myosin II’s motor activity. Surprisingly, these myosin II filaments are not stationary but dynamic; they do not stay along the cortex for a long time. With a half life time of 7.01 s, they are rapidly and continuously exchanged with others in the endoplasm. The dissociation requires disassembly of filaments induced by MHC phosphorylation. Dissociated myosin II is released from the cortex. The released myosin II is dephosphorylated by the MHC phosphatase in the endoplasm. Then, dephosphorylated myosin II assembles into filaments and becomes associated with cortical actin filaments again. Although individual myosin II molecules keep changing their location between the endoplasm and the cortex cyclically, as a mass, they gradually flow along the cortex during contractile ring formation and cytokinesis. To elucidate the detailed mechanism of myosin II dynamics, it will be necessary to directly observe the dynamic feature of filaments or single molecules in a live cell in the future.

Materials and methods

Cell culture
S65T GFP-tagged MHC gene was expressed extrachromosomally in MHC-null cells as previously described (Moores et al., 1996; Yumura and Uyeda, 1997b). 3ALA MHC gene was originally constructed by Egelhoff et al. (1993), and tagging with wild-type GFP was performed by Dr. Uyeda (National Institute for Advanced Interdisciplinary Research). Cells were cultured in a nutrient medium (HL5) supplemented by 10 μg/ml G418 (Geneticin; Sigma-Aldrich) in shaking condition (115 rpm) or in culture dishes. Cells were washed with BSS (10 mM NaCl, 10 mM KCl, and 3 mM CaCl2) and incubated in the same solution for 2–5 h before experiments. The removal of HL5 medium was necessary because the medium emitted fluorescence when illuminated by argon laser (488 nm).

Release of myosin II from Triton ghosts
Triton ghosts were prepared as described (Yumura, 1991). In brief, cells were treated with a buffer containing 10 mM Pipes (pH 7.5), 5 mM EGTA, 5 mM EDTA, 20 mM KCl, 1 mM DTT, 0.2 mM phenyl methyl sulfonyl fluoride, and 0.5% Triton X-100. After 7 min, lysed cells were collected by centrifugation and washed with a buffer containing 10 mM Pipes (pH 7.5), 50 mM KCl, 2 mM MgCl2, 1 mM DTT, and 0.2 mM phenyl methyl sulfonyl fluoride. Lysed cells were mainly composed of cortical cytoskeletons (Yumura, 1991). To quantify the release of myosin II from Triton ghosts, 1 mM ATP was added to the suspension of Triton ghosts, and after centrifugation, the supernatant (released fraction) was charged on SDS-PAGE after acid precipitation. The gels were stained with Coomassie blue. Relative amount of myosin II was measured from the density of the bands corresponding to MHCs. The phosphorylation of released MHC from the Triton ghosts was examined by adding γ[32P]ATP in 1 mM ATP as described previously (Yumura and Kitaniishi-Yumura, 1992).

Figure 10. Schematic summary of myosin II dynamics during contractile ring formation. (A and B) Two models for recycling myosin II between the cortex and the endoplasm. Red bars represent cortic-ces. In model A, monomers form filaments in the cortex. The association between myosin II and the cortex is dependent on the state of MHC phosphorylation. In model B, filaments are formed in the endoplasm and become associated with the cortex by an unknown mechanism. (C) Overview of dynamic cortical flow model during contractile ring formation. Some myosin II filaments are associated with cortical actin (red zone) and move toward the equator (cortical flow). The accumulated filaments produce force for ATP-dependent contraction of the contractile ring. These filaments disassemble into monomers via heavy chain phosphorylation and are released into the endoplasm. The released monomers are dephosphorylated, assemble into filaments in the endoplasm and become associated with the cortical actin again.
Confocal microscopy

Cells were placed on a coverslip (18 × 18 mm; No. 1) and overlaid with a thin agar sheet as described previously (Yumura and Fukui, 1985). The coverslip was inverted and placed over a glass slide attached with a support made of two-sided adhesive tape (5 mm wide, 0.3 mm thick). The sample was then sealed with plastic glue (Fastening Systems).

An inverted microscope (Axiorent 135M, Carl Zeiss, Inc.) was equipped with a confocal laser system (LSM 510, Carl Zeiss, Inc.). An oil immersion plan fluor 100× objective lens (NA 1.3) was used. Argon laser (488 nm, 25 mW) was selected for time-lapse acquisition of images. Sequential images were captured as stack images by a time series imaging software (Carl Zeiss, Inc.). The duration for a single image acquisition was 30–90 ms, and intervals were usually 30 ms. To prevent photobleaching during image acquisition, the intensity of the excitation light was set at the minimum value. The actual decrease of the fluorescence intensity during a series of experiments (typically 1 min) was less than 1–2%, which did not affect cell behavior and motility.

Photobleaching

The confocal laser system (LSM 510) was able to bleach fluorescence of a selected area. For photobleaching, the full power of argon laser (488 nm and 514 nm lines) was applied. Usually 5 times iterated illumination was required for >95% photobleaching. Images were acquired at 12 bits depth with 1024 × 1024 pixels. The changes of fluorescence intensity in the bleached area was monitored in respect to time after background subtraction. These data were applied to curve fitting using the Sigma Plot software (Jandel Corp.). The time course of recovery was fitted to the following equation for a single exponential rise to maximum:

\[ Fr = a \left[ 1 - \exp(-bx) \right] + c, \]

where, \( a \) is the amplitude of exponential, \( b \) is the rate constant, \( x \) is time (in seconds), and \( c \) is a constant. To assess the quality of fitting, log(a + c − Fr) was plotted versus x in Fig. 1 C. The half time for recovery (\( t_{1/2} \)) was calculated using the following equation:

\[ t_{1/2} = -\ln\left(0.5 + c/(2a)\right)/b. \]

For FLIP, the time course of fluorescence loss was fitted to the following equation for a simple exponential decrease to minimum:

\[ Fl = a \left[ \exp(-bx) \right] + c, \]

where \( a \) is the amplitude of exponential, \( b \) is the rate constant, \( x \) is time (in seconds), and \( c \) is a constant.

Online supplemental material

The QuickTime video (Video 1), which clearly shows cortical flow of myosin II toward the equator (see details in legend to Fig. 9), can be found at http://www.jcb.org/cgi/content/full/200101103/DC1.

I would like to thank Dr. T.Q.P. Uyeda for providing me with GFP–myosin II and GFP–3ALA myosin II, and for his helpful discussions. I also thank Dr. T. Kitanishi-Yumura for critical discussions, Mr. Go Ito for allowing me to use unpublished data, and Ms. N. Sherwood for proofreading.

This research is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Submitted: 2 November 2000
Revised: 30 May 2001
Accepted: 5 June 2001

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