A *Dictyostelium* Myosin II Lacking a Proximal 58-kDa Portion of the Tail is Functional In Vitro and In Vivo*

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We used molecular genetic approaches to delete 521 amino acid residues from the proximal portion of the *Dictyostelium* myosin II tail. The deletion encompasses ~40% of the tail, including the S2-LMM junction, a region that in muscle myosin II has been proposed to be important for contraction. The functions of the mutant myosin II are indistinguishable from the wild-type myosin II in our in vitro assays. It binds to actin in a typical rigor configuration in the absence of ATP and it forms filaments in a normal salt-dependent manner. In an in vitro motility assay, both monomeric and filamentous forms of the mutant myosin II translocate actin filaments at 2.4 $\mu\text{m/s}$ at 30°C, similar to that of wild-type myosin II. The mutant myosin II is also functional in vivo. Cells expressing the mutant myosin II in place of the native myosin II perform myosin II-dependent activities such as cytokinesis and formation of fruiting bodies, albeit inefficiently. Growth of the mutant cells in suspension gives rise to many large multinucleated cells, demonstrating that cytokinesis often fails. The majority of the fruiting bodies are also morphologically abnormal. These results demonstrate that this region of the myosin II tail is not required for motile activities but its presence is necessary for optimum function in vivo.

INTRODUCTION

Myosins are the mechanochemical enzymes that convert the chemical energy of ATP hydrolysis to mechanical force that translocates actin filaments. Conventional myosin, or myosin II, has two heavy chains that fold into two globular head domains, each of which bind two light chains. Heavy chains emerge from each head and associate with each other in an alpha helical coiled coil forming an elongated tail domain (Figure 1). Work on muscle myosin II has shown that the distal part of the tail, called light meromyosin (LMM), is required for the assembly of myosin thick filaments (Lowey *et al.*, 1969). These thick filaments are present in both muscle and nonmuscle cells and are believed to be involved in various motile activities (for review, see Spudich, 1989).

The function of the proximal part of the tail, called subfragment 2 (S2), remains controversial. It has been proposed that the S2 region is a molecular spring that confers the elasticity of the system (Huxley and Simmons, 1971) and that a hinge at the S2-LMM junction may be a flexible joint allowing the heads to efficiently

interact with actin filaments (Huxley, 1969). This S2-LMM hinge region also has been proposed by Harrington (1971, 1979) to play an active role in force production. He proposed that local helix-coil transitions in the S2-LMM hinge region is the origin of contractile force. This hypothesis is supported by inhibition of production of maximum force by an antibody specific to S2 (Harrington *et al.*, 1990; Sugi *et al.*, 1992). Such a suggested role would indicate that this region of the tail should be critical for myosin II function both in vitro and in vivo. Opposed to this idea are data demonstrating that subfragment 1 (S1), the head region of myosin, is sufficient to generate movement of actin filaments in vitro (Toyoshima *et al.*, 1987) and to produce force in vitro (Kishino and Yanagida, 1988). However, the speed of movement by S1 is slower than full-length myosin II in vitro and it is not clear whether the force produced by S1 in vitro is the same as in vivo, leaving the possibility that S2 is needed for optimum function.

Here we investigate the function of the region of the *Dictyostelium* myosin II tail that has positional correspondence to the proximal region of the muscle myosin II tail, including the S2-LMM junction domain. Like muscle myosin II, the *Dictyostelium* myosin II tail can be divided proteolytically into a proximal soluble fragment and a distal insoluble fragment. This cleavage

* We dedicate this manuscript to the memory of Dr. William F. Harrington, who made seminal contributions to the understanding of the mechanism of muscle contraction and who inspired the work of many others in this field.

point in *Dictyostelium* myosin II is similar in position to that in muscle myosin II (Figure 1). Furthermore, the overall domain structure of the *Dictyostelium* myosin II tail is very similar to that of muscle myosin II, with the exception of an additional phosphorylation domain for the regulation of assembly at the C-terminus of the *Dictyostelium* myosin II tail (Figure 1). We therefore use the terms *Dictyostelium* S2 and LMM to refer to the regions in the *Dictyostelium* tail that correspond to those in the muscle myosin II tail. However, we do not wish to imply that the precise *in vivo* functions of these regions in muscle and in *Dictyostelium* are necessarily the same. For example, it has not been demonstrated that the *Dictyostelium* myosin II has a flexible hinge structure, as suggested for the muscle myosin II tail, near the S2-LMM junction.

The *Dictyostelium* system has well-established methodologies that allow expression of genetically engineered mutant myosin IIs and biochemical isolation of the myosin IIs for *in vitro* characterization (Egelhoff *et al.*, 1991; Patterson *et al.*, 1991). *Dictyostelium* also has the advantage of having several well-characterized phenotypes that result from defects in myosin II function *in vivo* (DeLozanne and Spudich, 1987; Knecht and Loomis, 1987; Manstein *et al.*, 1989). Therefore, we have created a mutant *Dictyostelium* myosin II that has a deletion of the proximal portion of its tail corresponding in muscle myosin II to the S2-LMM hinge and ~70% of the S2 region. Here we report both the properties of this purified mutant protein and the *in vivo* phenotypes of a strain carrying this mutant myosin II in the absence of the wild-type myosin II.

MATERIALS AND METHODS

Generation of the Mutant Myosin II

Standard methods were used for all DNA manipulations (Sambrook *et al.*, 1989). pMyDAP is a pTZ18R-derived plasmid bearing the entire *Dictyostelium* myosin II coding sequence (Egelhoff *et al.*, 1990). This plasmid was digested with *Sna*BI and religated, resulting in a deletion of amino acid residues 934–1454. The *Bst*XI-*Kpn*I fragment of this plasmid was used to replace the wild-type *Bst*XI-*Kpn*I fragment in pSB2, an extrachromosomal hygromycin-resistance vector bearing full-length wild-type *Dictyostelium* myosin II coding sequence fused with the actin 15 promoter and terminator (Egelhoff *et al.*, 1989). The resulting plasmid pEK1 was transformed by electroporation (Egelhoff *et al.*, 1991) into a myosin II null mutant HS2206 that was generated from the wild-type Ax2 cell line by gene replacement (Manstein *et al.*, 1989). Cell lines resistant to 30 µg/ml hygromycin were isolated, and expression of the mutant form of myosin II (MyΔ934–1454) was checked by analysis of the whole cell lysates by western blotting using My4, a monoclonal antibody that binds to the distal LMM portion of *Dictyostelium* myosin II heavy chain (Peltz *et al.*, 1985).

In an independent experiment, MyΔ934–1454 gene was cloned into pBIG (Patterson, personal communication), a G418 resistance extrachromosomal vector derived from pATANB43 (Dynes and Firtel, 1989). The resultant plasmid was transformed into G418-sensitive myosin II null cells, which were obtained by gene replacement using thymidine kinase as a marker in thymidine auxotroph cells (Ruppel, unpublished data). Four independent transformants were isolated in the presence of 8 µg/ml G418.

Growth and Development

The wild-type Ax2 cells and the mutant cells expressing MyΔ934–1454 in place of the native myosin II were maintained as suspension cultures in HL5 media (Sussman, 1987). Myosin II null cells were maintained on petri dishes containing HL5 media.

To examine the ability of the above cell types to undergo cytokinesis, cells were grown in suspension in HL5 on a rotary shaker at 22°C. Cell density was determined every 12 h using a hemacytometer. The developmental phenotypes of each cell line were examined by spotting small aliquots of cell suspension on a lawn of *Klebsiella aerogenes* on SM/5 plates (Sussman, 1987). After several days at 22°C, formation of fruiting bodies was assessed under a dissection microscope.

Protein Purification

Myosin IIs were purified by a method of Uyeda and Ruppel (unpublished data). In brief, *Dictyostelium* cells expressing either the wild-type myosin II or MyΔ934–1454 were grown in HL5 media on a rotary shaker at 22°C. All procedures thereafter were performed at 0–5°C, and all the solutions contained 1–5 mM dithiothreitol (DTT). Cells were harvested, washed, and lysed by sonication in a buffer that contained 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, 50 mM NaCl, and 2 mM EDTA and a cocktail of proteinase inhibitors. The suspension of lysed cells was centrifuged at 18 000 rpm for 30 min in a Beckman (Fullerton, CA) JA20 rotor, and myosin II was extracted from the pellet by homogenization in a buffer that contained 10 mM HEPES, pH 7.4, 250 mM NaCl, 3 mM MgCl₂, and 2 mM ATP. The extract was diluted fivefold with buffer HE (10 mM HEPES, pH 7.4 and 1 mM EDTA) and spun at 50 000 rpm for 1 h in a Beckman 70Ti rotor. The pellet was homogenized in a buffer that contained 10 mM HEPES, pH 7.4, 500 mM NaCl, 3 mM MgCl₂, and 2 mM ATP. The homogenate was clarified by centrifugation at 50 000 rpm for 5 min in a Beckman TL100.3 rotor, diluted 10-fold with buffer HE, and spun at 50 000 rpm for 1 h in a 70Ti rotor. This final pellet was dissolved in buffer HE that contained 500 mM NaCl and was used as the purified myosin II fraction.

Rabbit skeletal muscle actin was purified by the method of Spudich and Watt (1971).

Salt Dependence of Filament Assembly

Purified solutions of myosin II in buffer HE containing 500 mM NaCl were diluted to ~250 µg/ml with the same buffer and dialyzed against a solution of 4 mM HEPES, pH 7.4, and 1 mM EDTA. Aliquots (30 µl) of the dialyzed myosin II were mixed with 30 µl of NaCl solutions of appropriate concentrations, incubated for 10 min on ice, and sedimented at 55 000 rpm for 10 min at 5°C in a Beckman TLA 100 rotor. The supernatants and the pellets were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The amount of myosin II in each fraction was quantitated by scanning the gel with a gel scanner (Ultrascan XL, Pharmacia LKB, Uppsala, Sweden) after staining with Coomassie Brilliant Blue, using wild-type *Dictyostelium* myosin II as a standard.

Electron Microscopy

Samples of the mutant myosin II and the wild-type myosin II (1 mg/ml) in 10 mM HEPES, pH 7.0, 500 mM NaCl were diluted with 9 volumes of 10 mM HEPES, pH 7.0, 1 mM MgCl₂, and 1 mM ATP and sedimented at 50 000 rpm for 10 min at 5°C in a TLA 100 rotor (Beckman). The myosin II filaments were resuspended in 1 M ammonium acetate, in which myosin II remained in filamentous form. To obtain monomeric myosin II, the myosin II filaments were resuspended in 1.5 M ammonium acetate. Samples were diluted to 50 µg/ml in 70% glycerol and rotary shadowed as described (Flicker *et al.*, 1985). For negative stain microscopy, samples of actin (50 µg/ml) and myosin II (100 µg/ml) were mixed in a buffer (25 mM imidazole-Cl, pH 7.4, 250 mM KCl, 4 mM MgCl₂, 1 mM DTT) and applied to carbon-coated nitrocellulose grids for 30 s followed by 1% uranyl

Figure 1. Schematic representations of myosin II of rabbit skeletal muscle and of *Dictyostelium*. The top line shows the relative scale of the two myosin II tails. The large arrows point to the region susceptible to chymotrypsin cleavage, which defines the S2-LMM junction in muscle myosin II (Lowey *et al.*, 1969) and in *Dictyostelium* myosin II (Pagh *et al.*, 1984; Flicker *et al.*, 1985; Truong *et al.*, 1992; Flicker, personal communications). Hatched bars show minimum domains that are demonstrated to be sufficient for filament assembly in vitro for human skeletal muscle myosin II (Sohn, Vikstrom, Szent-Gyorgyi, and Leinwand, unpublished data) and for *Dictyostelium* myosin II (Pagh *et al.*, 1984; DeLozanne *et al.*, 1987; O'Halloran *et al.*, 1990).

acetate stain. Specimens were examined with Philips EM201 and EM400 electron microscopes (Philips Electronic Instruments, Mahwah, NJ).

In Vitro Movement Assay

The motile activity of *Dictyostelium* myosin II is regulated by phosphorylation of the regulatory light chain (Griffith *et al.*, 1987). For the in vitro movement assay, therefore, purified myosin II (750 μ g/ml) was treated with bacterially expressed *Dictyostelium* myosin II light chain kinase (25 μ g/ml) that lacks the autoinhibitory domain (Tan and Spudich, 1991) in 10 mM HEPES, pH 7.4, 50 mM NaCl, 3 mM $MgCl_2$, 2 mM ATP, 1 mM DTT overnight on ice (Ruppel, unpublished data). Phosphorylated myosin II was sedimented at 70 000 rpm for 10 min in a Beckman TLA 100.2 rotor at 4°C, dissolved in 10 mM HEPES, pH 7.4, 200 mM NaCl, 3 mM $MgCl_2$, 2 mM ATP, and 1 mM DTT, mixed with 0.1 mg/ml rabbit skeletal muscle actin, and sedimented at 50 000 rpm for 10 min at 4°C in a TLA 100 rotor. This procedure removes denatured myosin II that binds to actin filaments in an ATP-insensitive manner (Kron *et al.*, 1991). The concentration of myosin II in this solution was determined by scanning SDS acrylamide gel lanes, as described above.

In vitro assay of movement of fluorescently labeled actin filaments over nitrocellulose surfaces coated with myosin II was performed at 30°C according to the method of Uyeda *et al.* (1990), which was a modification of the earlier methods (Kron and Spudich, 1986; Toyoshima *et al.*, 1987). The major modification was the addition of 0.8% methylcellulose in the motility assay buffer (25 mM imidazole-Cl, pH 7.4, 25 mM KCl, 4 mM $MgCl_2$, 1 mM ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 2 mM ATP, 10 mM DTT), which enables one to observe movements of actin filaments over a wide range of myosin densities. The average sliding velocity of actin filaments was measured using a video micrometer with a video frame counter (Sheetz *et al.*, 1986). Filaments whose lengths were between 2 and 4 μ m and that moved continuously >10 μ m were scored.

RESULTS

Expression of MyΔ934-1454

The plasmid pEK1 contains a hygromycin resistance gene, an origin of replication in *Dictyostelium*, and the

mutated *Dictyostelium* myosin II heavy chain gene. The mutant heavy chain gene has a deletion that removes 521 amino acid residues, fusing leucine 933 to arginine 1455. This deletion corresponds to ~40% of the *Dictyostelium* myosin II tail and includes cleavage sites by chymotrypsin that have been located within this region (Pagh *et al.*, 1984; Flicker *et al.*, 1985; Truong *et al.*, 1992) (Figure 1). The chymotryptic cleavage sites are within a similar region in muscle myosin II and define the S2-LMM junction (Lowey *et al.*, 1969). Thus, this deletion in the *Dictyostelium* myosin II tail includes the S2-LMM junction as well as the majority of the S2 region.

The plasmid was electroporated into HS2206 myosin II null cells (Manstein *et al.*, 1989). Clonal transformants were selected by resistance to hygromycin, and the expression of the MyΔ934-1454 was verified by immunoblot analysis using a monoclonal anti-*Dictyostelium* myosin II antibody (Figure 2, C and D). The molecular weight of the MyΔ934-1454 heavy chain was estimated to be 180 kDa, which is consistent with the deletion of 521 amino acid residues from the 240-kDa wild-type heavy chain. The level of expression of this protein is comparable with that of the wild-type myosin II in Ax2 wild-type cells (Figure 2, C and D).

Purification and Solubility of MyΔ934-1454

Wild-type and MyΔ934-1454 myosin IIs were purified as described in MATERIALS AND METHODS (Figure 2, E and F). Typically, ~0.1 mg of the protein per gram of cells was obtained.

Wild-type *Dictyostelium* myosin II is soluble at high salt concentrations but assembles into insoluble fila-

Figure 2. SDS-polyacrylamide gel and immunoblot of whole cell lysates and purified myosin IIs. Whole cell lysates of wild-type cells (A and C) and of My Δ 934-1454 mutant cells (B and D) were electrophoresed on a 7.5% gel and probed with anti-*Dictyostelium* myosin II antibody (C and D) or stained with Coomassie Brilliant Blue (A and B). Each lane was loaded with 2 μ g of protein. Purified wild-type myosin II (E) and My Δ 934-1454 (F) were electrophoresed on the same gel and stained with Coomassie Brilliant Blue.

ments at low salt concentrations (Clarke and Spudich, 1974). The solubility of the mutant myosin II was compared with the wild-type myosin II at different salt concentrations (Figure 3). At concentrations of 0, 50, 100, and 200 mM NaCl, there were no significant differences between the mutant myosin II and the wild-type myosin II in the amount of myosin II in the pellet after high-speed centrifugation.

Electron Microscopy

My Δ 934-1454 forms thick filaments that are somewhat shorter than those made from the wild-type myosin II (Figure 4, C and D). Similarly, the tails of individual My Δ 934-1454 monomers are 118 ± 10 nm (mean $\pm$ SD; $n = 20$) long, 68 nm shorter than that of the wild-type myosin II (Figure 4, A and B), which is 186 nm long (Flicker *et al.*, 1985). The difference in length is consistent with the deletion of 521 amino acid residues, assuming that the rise between alpha carbons in the coiled-coil tail is 0.148 nm (Crick, 1953).

Negative staining of myosin II samples demonstrates that the My Δ 934-1454 can bind to actin filaments in an apparently typical rigor configuration (Figure 5). This decoration occurs only in the absence of ATP.

In Vitro Movement Assay

In further pursuit of a possible functional defect of the My Δ 934-1454, we compared the velocities of actin filaments sliding over nitrocellulose surfaces coated with either the wild-type myosin II or the My Δ 934-1454. Because it was plausible that the defect may be apparent only when myosin II formed filaments, each myosin II

was bound to the nitrocellulose surfaces either in 200 mM NaCl when $>85\%$ of the myosin II should be in a monomeric form or in 50 mM NaCl when $>90\%$ of the myosin II should be in a filamentous form (Clarke and Spudich, 1974) (Figure 3).

The sliding velocity in our in vitro movement assay depends on the density of myosin II heads on the surface and reaches an asymptote at a certain density (Uyeda *et al.*, 1990). Therefore, we assayed movements at several different myosin II densities for each condition. The sliding velocity reached the asymptote when nitrocellulose surfaces were coated for 2 min with myosin II solutions of 70 (My Δ 934-1454) or 85 (wild-type myosin II) μ g/ml, and application of more myosin II resulted only in more fragmentation of actin filaments. The movements over surfaces treated with 70 (My Δ 934-1454) or 85 (wild-type myosin II) μ g/ml myosin II solutions were generally smooth and $>90\%$ of the actin filaments moved continuously. The average sliding velocities of these movements are shown in Table 1. There was no significant difference in the asymptote speeds between the filamentous form and the monomeric form within each type of myosin II or between the wild-type myosin II and the My Δ 934-1454 within each assembly state of myosin II.

Growth and Development of My Δ 934-1454 Cells

Cells expressing the My Δ 934-1454 can grow and divide in suspension culture in HL5 media, unlike the parent cell line of HS2206 (a myosin II null mutant), which is unable to divide and becomes multinucleated in suspension (Manstein *et al.*, 1989). However, My Δ 934-1454 cells grown in suspension culture show a wide range of cell sizes compared with wild-type cells (Figure 6, A and C). The large cells are multinucleated (Figure 6C, inset), indicating that cytokinesis frequently fails to

Figure 3. Dependence of myosin II solubility on salt concentrations. Purified wild-type myosin II (▨) and My Δ 934-1454 (■) were incubated in buffers containing varying concentrations of NaCl from 0 to 200 mM, and the percent of protein that was soluble after ultracentrifugation was quantitated by scanning SDS acrylamide gels.

Figure 4. Rotary shadowed myosin II molecules. Myosin II molecules in solution were sprayed on mica and rotary shadowed. My Δ 934–1454 in monomeric form (A). Wild-type myosin II in monomeric form (B). My Δ 934–1454 in filamentous form (C). Wild-type myosin II in filamentous form (D). Magnification, 144 000 $\times$.

occur after karyokinesis. This may explain why the My Δ 934–1454 cell line multiplies slower than the wild-type Ax2 cells (Figure 7).

The formation of fruiting bodies depends on myosin II function, and the development of the myosin II null cells (HS2206) is arrested at the mound stage (Manstein *et al.*, 1989). In contrast, the My Δ 934–1454 cells aggregate and proceed through further developmental stages (Figure 8C). Some aggregates form normal fruiting bodies, but many form unusually small sori in which spores are encapsulated. Other aggregates in the population form thick stalk-like structures without noticeable sori.

This developmental phenotype is not specific to the particular isolate we used in this study, since two other independent cell lines transformed with pEK1 behaved in the same way. In addition, this phenotype was observed in My Δ 934–1454 cells generated using a different construct and a different myosin II null cell line. In that experiment, the My Δ 934–1454 gene was cloned into pBIG, a G418 resistance extrachromosomal vector. The

resultant plasmid was transformed into G418-sensitive myosin II null cells. All four independent transformants produced fruiting bodies but many of them had very small or no sori, resembling those shown in Figure 8C.

DISCUSSION

We have demonstrated that the deletion, which covers much of the S2 region and the S2-LMM junction, does not measurably impair the functions of the molecule in our *in vitro* assays. In previous studies using *Dictyostelium* or rabbit skeletal muscle myosin II, it has been shown that S1 (the head fragment of myosin II) alone can support movement but at a significantly slower rate (about one-third) compared with the full-length myosin II or heavy meromyosin (HMM; two S1 heads bound to S2) (Toyoshima *et al.*, 1987; Manstein *et al.*, 1989). The double-headed nature of myosin II or HMM cannot explain the faster rate of movement since single-headed

Figure 5. Actin filaments decorated with My Δ 934–1454. Rabbit skeletal muscle actin filaments complexed with My Δ 934–1454 in the absence of ATP were applied to carbon coated electron microscope grids and negatively stained with uranyl acetate. Magnification, 142 500 $\times$.

Table 1. Speed of actin filaments in an *in vitro* movement assay^a

Myosin II type	Monomers ^b ($\mu\text{m/s}$)	Filaments ^c ($\mu\text{m/s}$)
Wild-type	1.88 ± 0.16	2.27 ± 0.21
My Δ 934–1454	2.35 ± 0.21	2.39 ± 0.17

^a Data are shown as average of 50 filaments $\pm$ SD.

^b Myosin IIs were deposited on the microscope cover slip in a buffer that contained 200 mM NaCl, which kept >85% of the myosin II in a soluble form.

^c Myosin IIs were incubated in a buffer that contained 50 mM NaCl for 5 min on ice before being deposited on the microscope cover slip. More than 90% of the myosin II was in filamentous form after this incubation.

Figure 6. Cells in suspension culture. Wild-type cells (A), myosin II null cells (B), and My Δ 934–1454 cells (C) were grown in suspensions in HL5 media for 3 d, fixed with 1% formaldehyde, and photographed under a microscope (magnification, 100 $\times$). Inset of C is a fluorescence micrograph of My Δ 934–1454 cells stained with 1 μ g/ml diamidinophenylindole to show individual nuclei (magnification, 350 $\times$).

myosin II or HMM moves at a similar rate as the double-headed myosin II or HMM (Harada *et al.*, 1987; Toyoshima *et al.*, 1987). These observations indicated that S2 could be required for the normal maximum speed of movement in the *in vitro* motility assay. However, it was not clear whether S2 is actively involved in the force generation or it simply helps S1 to properly orient on the glass surface. In this study, we did not observe any significant difference in sliding velocities between the My Δ 934–1454 and the wild-type myosin II using either filamentous or monomeric myosin IIs (Table 1). This result suggests that the C-terminal majority of S2 and the S2-LMM junction region of *Dictyostelium* myosin II are not essential in some active manner for the maximum speed of the movement *in vitro* and that the slower movement by S1 *in vitro* can be best explained by preferential binding of a portion of the tail to the surface. This would leave S1 free to carry out its motor function more efficiently. We have not ruled out, however, that the undeleted short N-terminal region of S2 serves an active role in the maximum movement.

It should be noted that the force required to move actin filaments in our *in vitro* motility assay is almost negligible compared with the force that could be theoretically produced by a single muscle myosin II molecule (Oosawa, 1977; Kron and Spudich, 1986). Therefore, the results reported here do not demonstrate that My Δ 934–1454 produces comparable force to the wild-type myosin II.

We have shown both by electron microscopy and by solubility experiments that My Δ 934–1454 forms thick filaments. It is known that there is a domain in the LMM region of the muscle myosin II tail (Lowey *et al.*, 1969; Lu *et al.*, 1983) and in the *Dictyostelium* myosin II tail (Pagh *et al.*, 1984; DeLozanne *et al.*, 1987; O'Halloran *et al.*, 1990) that is alone required for the assembly of myosin II into filaments. Therefore, it is reasonable that the My Δ 934–1454, which contains the assembly do-

main, forms filaments in a normal salt-dependent manner (Figure 3).

The My Δ 934–1454 is also functional *in vivo* because it can partially rescue defects of myosin II null mutant cells. The mutant cells expressing My Δ 934–1454 can undergo cytokinesis and sporogenesis, albeit not as efficiently as wild-type cells. The lack of efficient myosin function is not due to a lower level of expression of the mutant myosin II compared with the normal level of expression of the wild-type myosin in wild-type cells (Figure 2). It is also not an artifact specific to this particular mutant cell line, because the same phenotype was reproduced in an independent cell line of a different background.

The inefficiency of My Δ 934–1454 *in vivo* could be due to some unknown structural or regulatory problems caused by the filaments of reduced length formed by

Figure 7. Growth of the cells in suspension. Wild-type cells (●), My Δ 934–1454 cells (▲), and myosin II null cells (◆) were grown on petri dishes in HL5 media for 24 h and were resuspended in HL5 media at a density of $\sim 1 \times 10^5$ /ml. Aliquots of each were counted every 12 h.

Figure 8. Development of fruiting bodies. Wild-type cells (A), myosin II null cells (B), and My Δ 934–1454 cells (C) were allowed to grow and develop on a lawn of *Klebsiella aerogenes* for 5 d at 22°C. Magnification, 18 $\times$.

the mutant myosin II (Figure 4). It is also possible that the domain we have deleted is required for generation of maximal force, as originally suggested by Harrington and his colleagues using rabbit skeletal muscle myosin II (Harrington *et al.*, 1990). Efforts are now underway to examine this point using My Δ 934–1454 in a laser trapping microscopy system coupled with the *in vitro* movement assay to directly measure force production by this molecule.

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