Multiple actin-based motor genes in *Dictyostelium*

Margaret A. Titus, Hans M. Warrick,
and James A. Spudich

Departments of Cell Biology and
Developmental Biology
Stanford University School of Medicine
Stanford, California 94305

Dictyostelium cells, devoid of conventional myosin, display a variety of motile activities, consistent with the presence of other molecular motors. The *Dictyostelium* genome was probed at low stringency with a gene fragment containing the conserved conventional myosin head domain sequences to identify other actin-based motors that may play a role in the observed motility of these mutant cells. One gene (*abmA*) has been characterized and encodes a polypeptide of ~135 kDa with a head region homologous to other myosin head sequences and a tail region that is not predicted to form either an alpha-helical structure or coiled-coil interactions. Comparisons of the amino acid sequences of the tail regions of *abmA*, *Dictyostelium* myosin I, and *Acanthamoeba* myosins IB and IL reveal an area of sequence similarity in the amino terminal half of the tail that may be a membrane-binding domain. The *abmA* gene, however, does not contain an unusual Gly, Pro, Ala stretch typical of many of the previously described myosin Is. Two additional genes (*abmB* and *abmC*) were identified using this approach and also found to contain sequences that encode proteins with typical conserved myosin head sequences. The *abm* genes may be part of a large family of actin-based motors that play various roles in diverse aspects of cellular motility.

Introduction

Cell biologists have long been fascinated by the wide variety of movements cells undergo, such as migration across a surface, rapid extension and retraction of lamellipodia and pseudopodia, cytoplasmic streaming, and rapid, saltatory movement of intracellular vesicles. The discovery of both actin and myosin in nonmuscle cells led to the attractive hypothesis that these proteins were involved in muscle-like force generating processes in cells. Myosin is capable of converting chemical energy in the form of ATP into mechanical work and has been intensively studied in both muscle and nonmuscle systems.

Myosins are broadly defined as a class of proteins that bind to actin in an ATP-sensitive manner and possess a MgATPase that is significantly activated by actin under physiological conditions (Pollard, 1981). Two distinct classes of myosin have been identified in nonmuscle cells. The first type of myosin, often referred to simply as myosin or as conventional myosin, is similar to its well-studied muscle counterpart. Conventional myosins are hexamers consisting of two heavy chains (~200 kDa) and two pairs of light chains (between 14 and 20 kDa). The C-terminal portion of the heavy chains form an alpha-helical coiled-coil and the N-terminal regions two globular heads. The tail region is involved in the formation of myosin thick filaments. The globular heads (often referred to as S-1) contain both an actin-binding and an ATPase site. S-1 alone is capable of generating movement in vitro (Toyoshima *et al.*, 1987).

The most striking difference between conventional myosins and the second type of myosins, referred to alternatively as unconventional, small, or myosin I, is that the latter are single-headed. Small myosins, first isolated from *Acanthamoeba* (Pollard and Korn, 1973) and subsequently from *Dictyostelium* (Cote *et al.*, 1985) and the intestinal brush border (Collins and Borysenko, 1984), are composed of a single heavy chain, varying from 110 to 140 kDa, and usually a single light chain (Korn and Hammer, 1988). The most notable exception to this composition is the brush border 110K protein which is complexed with three to four calmodulin molecules (see Mooseker, 1985 for a review). Sequence analyses of the N-terminal regions of small myosins reveals that they are homologous to the head region of conventional myosins, but the tail portions are divergent (Korn and Hammer, 1988). The characteristic hydrophobic repeats that lead to the coiled-coil structure of the conventional myosin tail are absent from the small myosin tail sequences, which may account for their single-headed structure (Hoshimaru and Nakanishi, 1987; Jung *et al.*, 1987).

The recent discovery of homologous recombination in *Dictyostelium* (De Lozanne and Spudich, 1987) and the existence of both conventional (Clarke and Spudich, 1974) and small (Cote *et al.*, 1985) myosins makes this an ideal organism in which to study the cellular role of the

Figure 1. Southern and northern analyses. (A) Hybridization of a *Dictyostelium mhcA⁻* head probe DNA to *Dictyostelium mhcA⁻* genomic DNA. For Southern analyses, each lane was loaded with 20 μ g of genomic DNA and digested with the appropriate enzyme. After electrophoresis in 0.8% agarose, the DNA was transferred to nitrocellulose and hybridized to the 32 P-labeled probe. The lengths of the fragments are given in kilobases (kb) and determined by electrophoresis of DNA fragments of known size in an adjacent lane. (B) Hybridization of *abmA* DNA to wild type *Dictyostelium* vegetative cell RNA. The lane was loaded with 10 μ g of vegetative cell RNA and electrophoresed in 1% agarose in the presence of formaldehyde. The RNA was then transferred to nitrocellulose and hybridized to the 32 P-labelled fragment. (C) Southern analysis of wild type *Dictyostelium* genomic DNA with the 3.3 kb Bgl II *abmA* probe.

1 TTAATAATAATAATCATGATTCGGATATTAAATTAATAATAAAAAAATAAAAAATGTTTCGTGATAAAAAAATAAAAAAATAAAAAAATAATCAGACTAACAGTTTTATTTTTATTTT
M

120 TTTTTTATTTTTATTTTTTAAACCAATTATGGGCAGAAATTTAAAGAGATTTAACTAAANNTGTTGGAGTTGAAGATTTAATTATGCTTACTGAAGTATCAGAATCTTCATTACATGAGA
A E F K R D L T K N V G V E D L I M L T E V S E S S L H E

240 ATTTAAAAATTAGATACAAAGAGGTTTAAATTTATGATGTAAAAAATAAAAAATAAAAAATAAATAAACAATAATAAATAATGATAATAATAAATAATGAATAAATAATGATAA
N L K I R Y K E G L I Y

360 AATAAAAAAGATCTCCTATTAAATTTATTAATTTTTTTTTTTTTATATTTTTATTTTTTTTTTAAAAATAATAAAAAAAACACATCAATCGGACCAGTATTAGTTTCAATGAATCC
T S I G P V L V S M N P

480 ATATAACAATTAGCAATTTATGCAATGATCAAAATTAATTTATATAAAGGTAAACATGAATTTGAAATTCACCACATATTTATAGTATTGCTGATAAAGCATATAGAGCATTAAAGATC
Y K Q L G I Y G N D Q I N L Y K G K H E F E I P P H I Y S I A D K A Y R A L R S

600 AGAGGGTGAAGATCAATGCTATTATATATCAGGAGAATCAGGAGCAGGTAAACAGAGCAAGTAATATATTATGCAATATTATGCAAGTATTACTGGTAGTAGTACAGAGGTAGAGAG
E G E N Q C I I I S G E S G A G K T E A S K Y I M Q Y I A S I T G S S T E V E R

720 AGTTAAAAACCAATTTTAAAGATCTAATCTTTATTCGGAAGCATTGGTATGGAAGAAACATTACGTAACAATAATTCAGTTCGTTTGGTAAATATATGEGAGATTCAAATTCATTTGGG
V K K T I L E S N P L A A E F G N G K T L R N N N S S R F G K A Y M E I Q F N L G

840 AGGTGATCCAGAAGGTGGTAAAAATACAAATACCTCTTGGAAAAGTCAAGTGTATCAATCAAACTCAAGGTGAACGTAATTTTCATATTTCTKATCAACTTTTAAAAAGTTCATCAGG
G D P E G G K I T N Y L L E K S R V I N Q T Q G E R N F H I F Y Q L L K G H Q G

960 AAAGAAAAATACAAATCTATTATCACCGGATCAATATCAATTTATTAAGTCAAGATGCAAGTAATGTTGGTTTTCTACTGCCAGATGGTATTGATGATCAAAATGGATTTAAACAAAAAA
K K T Y N L L S P D Q Y H Y L A T R N A S N G W F S L P D G I D D Q I G F K Q T K

1080 GAATGCAATGAAGGTGGTGGATCGATGCAACCAATTCCTTTGCAACATTTGCAAGTATTCAGCAATTTTACTCTGGGTAATCTTTCATCAATAAAATCCGCGCTCTGCTGAATGGTTC
N A M K V G I D E P L Q K K S F A T L S A I L L G N L S F N K S A S G T A N G S

1200 GGTAAATTCGGATAAAAAATAGCAAAATACAATAGCATCATTCATCGGTGTTGATGCAATAGTATTGGAAGGTCATTGGTATCTGTCAGATTAGTACAGGTCAAGGTGCACGTATTTG
V I S D K K L A N T I A S L M G V D A I V L E S S L V S R Q I S T G Q G A R I S

1320 AACCTATTGATACCAAAACCGTTGAACAGCTATGTATGCGCGTGTGCTATTGCTAAGCAACCTATAGTAAATTTATTTGATTTTCATCGTTAGAAAGATCAATCAATCCATTGAGT
T Y S V P Q T V E Q A M Y A R D A F A K A A T Y S K L F D F I V R K I N Q S I E V

1440 TAAACCAATTCGTAAGATTTGGTGTATTGCTATCTATGCTTTTGAATCTTTGGAACAATTCATTGCAACATTTGTCATCAATTTATGTCNAATGAAACCTTCAACAAATTTTCAT
K T I G K V I G V L D I Y G F E I F E N N S F E Q F C I N Y V N T E L Q Q I F I

1560 TGATCTAACATTCAAAACCTGAACAAGAGAAATACGTCCAAGAGCGGTATTACATGGATTCCAGTTCAATATATTAAATAAAGCATGTGTGATTAAATGAAAAGAGCGCAATTCGTAT
D L T L K T E Q E E Y V Q E G I T W I P V Q Y I N N K A C V D L I E K K P I G I

1680 TCTTTTCAATTTTGGATGAAGATGCTTTTCCCAGAAGGTAAATGATCAAATCTATGATCGATAAATTAATAAAGCACTTTTCAATCATACTCATTATTCAAAGGTAGAGAGACAAAAAGAA
L S L L D E E C L F P E G N D Q T M I D K L N K H F S N H T H Y S K V E R Q K N

1900 TTCACATTCATTATCAATCTATGCTGGTAAAGTTTCTATATATGATGGTTTCTCGATAAGAAATAGACATACACTTTAATGATTAGTTAGTACAGTCCAGGACAGTAGTAGTTC
S Q F I I N H Y A G K V F Y I D G F L D K N R D T L F N D L V T L A T S S C

2020 TTCCTATTGCTGCAAAATTTTCAATATGTACCACCACCTTGAAGTCGATCCAGAACAAGAGAAAAAGAAATCGTGATAAATTTTCAAGAAATGGTTTTGCAAAATATGCTGCAAAAAACATT
S L L V E I F K Y V P P L E V D P E Q E K K N R D K F S K N G F A N N A A K T F

2140 TATTCACAACCGATAAGAAAAGCCCAATCACTGCTGGTTTCCAAATTAAGAAATCAAGTTACTTCCTACTATTGAATACCTTTACAGTGTTCACCACATTATGTCGTTGTTATTAACCAAA
I P T D K K S P I T A G F Q F K N Q V T S L L K S L Y S C S P H Y V R C I K P N

2260 CTCNAATTCAGACAGATTCGAATGGGATCAAGTATGCTGCAACAGTGGCTTATCTGGCTCTTTTGAATAATTTAGTACGCTGGTGCAGGTTATGTTTCTGCTCAAACTTTCTCT
S N M R A L E W D Q S K A C A E Q V A Y L G S F E N L L V R R A G A Y C Y R Q T F S

2380 TAAATTTATGCGTGGTATTATATGATTGGTAAATCAACTTGGCCAAAAATGCTCAGGTGATGCTGAAAAAGGTGTTAATCTCTTAATGGGTGAATTAACCTCAAGAAATCAATCAAGAA
K F M R R Y Y M I G K S T W P K W S G D A E K G V N L L M G E L T Q E I N I K E

2500 GGAGGTTCAATATGGTAAAAACAAGATTTTTCATTCGTAATCCACAACCAATGTTCTTATTAGAGGATAAAGAAATAAGAGATTAAATGATTAGCCACTAAAAATGGATCAGTTTGGAA
E V Q Y G K T K I F I R N P Q P L F L L E D K R N K R L N D L A T K I G S V W K

2620 AATGATAAACAAGAAATGGTATTACGTACTTTAGCTGCAATTAATTAACCAAGGACCTATAGAGGTTGTTTACTGGTTAGAGAACTGTTTAAACTAAAGAAATCAATCAATAGTAT
M Y K Q G R K W Y L R T L A A I K I Q R T Y R G G W L V R E C V K L K N Q S I S I

2740 TTTCAAAAATAAAGAGAGAAATAGACAAATCAATTAACCTTAGTAAAGCTGCTTATTGGTGATTTCCTCTCACTCACAAGAGATAAATATACCAAGGACGCTTACCCAGCGGAAG
F Q N N K E R N R Q S I K L S K A A F I G D F L S L T R D N Y T T D A L P S E E

2860 AGGTCAAAATGACTGTTCTTCGAAGCGCTCTTTAAATGTTTCCAAAGTTAGTCGTCATCATAGATTCAAAAACGTTCAATATTAGTTACCAATGAACCATTTTCAAAATTAGACCAC
G P N D C S S K R S L N V S K V S R H H K I Q K R S L L V T N E I T T C T I T T F H

2980 TAAACCAAGAAAGAGATGGTTTTCGCAATTAACRGTAAAGTGGCCCTCTCTGCAATTAAGAAAAATTCATTGAGTAACTCTGATGACTTTTTCGTTATTCATATCAATCAATGAA
K P K K D G S W F A I A K R K V P F S A I E K I S F S K L S D D F F V I H I I N E

3100 ACATGATCTCTGTTTGGAAACCAATAAAAAACTGTATTAAATACCTCTTATCAAAATTTATTTCAAAAACATTAAATGCTAAAGAAATGGTTTTGAAATTCAGAGATTCATTCAATTA
H D L C L E T N K K T V L I T L L S N L Y S K H L N G K E L V F E F K D S I Q Y

3220 TCGTAATCAAAAAGGTCTCTCAGAAATGAATTTGTAAAGGTTGATTCAATTCATGAAAAATCTTCAAAATTCACCTCAAGCTAACTCTCTCTTTTACCTGCAAAAGCAGAAAAAGATTA
R N Q K G P S E L K F V K V D S I H E K S S N S P Q A N S P S F T A K A E K N Y

3340 TTTAAAAAGTTTGTGTTTAACTGGTCTTTCTGCAATCAAAATCTATTTTAAATGAAAAATAAATCTTTTTTTTTTAAACAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA
L K V C V L P G L S S E S K S I L I E K *

3460 GGAATTCCTTATTATTTGATTATAATATTATATAAAAAAGTAA

Figure 2. Nucleic acid sequence and deduced amino acid sequence of *abmA*. The single-letter code is used to indicate the amino acids. The underlined amino acids are residues that are located in the highly conserved regions of the myosin head. The numbers on the left refer to the position of the nucleotide starting each line in the sequence. Underlined bases indicate splice acceptor/donor sites.

actin-based motors. The single gene encoding the conventional myosin, *mhcA*, has been cloned and sequenced (De Lozanne *et al.*, 1985; Warrick *et al.*, 1986) and its *in vivo* function clarified using molecular genetic techniques (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Manstein *et al.*, 1989). *Dictyostelium* cells that have had the conventional myosin gene altered or removed are defective in cytokinesis but are still capable of a wide range of motile behaviors, such as migrating across a substrate and extending and retracting pseudopodia and lamellipodia (Peters *et al.*, 1988; Wessels *et al.*, 1988; Manstein *et al.*, 1989). These results have prompted speculation that other actin-based motor proteins, such as small myosins, may participate in these motile processes. We have undertaken a broad search for genes encoding actin-based motors in *Dictyostelium* using a probe from the highly conserved region of the myosin head. A preliminary account of this work has been presented (Titus *et al.*, 1988).

Results

The availability of a strain of *Dictyostelium* lacking the single high molecular weight conventional myosin gene (Manstein *et al.*, 1989) facilitated the search for actin-based motor genes using DNA hybridization under low stringency conditions. Southern analysis of genomic *mhcA*[−] DNA was performed using a 1.8 kb fragment derived from the head region of the *mhcA* gene (Warrick *et al.*, 1986). This probe, which contains all of the highly conserved regions of the myosin head, was chosen to detect a broad spectrum of genes similar to myosin. The probe hybridizes to a discrete series of bands in each restriction digest (Figure 1A). Fragments of 9 kb and 3.3 kb are visible in a *Bcl* I digest and also in a *Bgl* II digest. The *mhcA* probe also hybridizes to a single 7.5 kb *Eco*RI fragment, an 8 kb *Hind* III fragment and both 10 kb and 3.3 kb *Pst* I fragments. The same pattern of hybrid-

ization was obtained when myosin head region probes from a variety of sources, such as *Acanthamoeba* myosin IB (Jung *et al.*, 1987) and lambda 3.5 (Horowitz and Hammer, 1988), *Drosophila ninaC* (Montell and Rubin, 1987) and *C. elegans unc-54* (MacLeod *et al.*, 1981) were used (data not shown).

The *Dictyostelium mhcA* probe was used to screen lambda genomic libraries generated from *mhcA*[−] DNA. The first clone identified, referred to as *abmA* (actin-based motor), from the EMBL3 *Bgl* II library contained a 3.3 kb *Bgl* II fragment that hybridized to the probe. This *Bgl* II fragment was excised, cloned into pTZ-18R and sequenced. The nucleic acid sequence of *abmA* (Figure 2) contains two introns at the 5' end of the gene. The introns are A-T rich, as is typical of *Dictyostelium*, and contains the expected splicing signals. The 3' end of the *abmA* gene contains termination and polyadenylation signals.

Northern analysis of *abmA* reveals that it is expressed in vegetative cells. The mRNA transcript is 3.8 kb (Figure 1B), which indicates that the *abmA* gene encodes for a polypeptide with a molecular weight of ~135 000.

Analysis of the deduced amino acid sequence of the coding region reveals the presence of segments of general myosin homology in the N-terminal portion of *abmA* (underlined in Figure 2). The sequence of the *abmA* head region was compared to available myosin head sequences. A detailed comparison over two regions of the *abmA* head with *Dictyostelium* myosin I (Jung *et al.*, 1989), *Acanthamoeba* myosins IL and IB (Jung *et al.*, 1987, 1989), bovine intestinal M1hc (Hoshimaru and Nakanishi, 1987), *mhcA* (Warrick *et al.*, 1986), and *C. elegans unc-54* (Karn *et al.*, 1983) is shown in Figure 3. The absolutely conserved G-E-S-G-A-G-K-T-E putative ATP-binding sequence (region I), typical of all myosins characterized to date, is present (Figure 3). The actin binding region (region II) is also present although there are a greater number of conservative sub-

Figure 3. Comparison of the deduced amino acid sequence of *abmA*, *abmB*, and *abmC* with myosin head sequences. (A) Schematic representation of the *abmA* gene product with the location of regions I and II in the linear sequence indicated. The stippled region represents the myosin head and the open box the tail of the molecule. The sequence of 2 highly conserved regions of the myosin head are shown, region I (ATP binding) includes residues 178 to 208 and region II (actin-binding region) includes residues 710 to 760. Numbering of the residues is taken from Warrick and Spudich (1987) and the single-letter amino acid code is used. Dictymyo 1, *Dictyostelium* myosin I (Jung *et al.*, 1989); Acanmyo 1L, *Acanthamoeba* myosin IL (Jung *et al.*, 1989); Acanmyo 1B, *Acanthamoeba* myosin IB (Jung *et al.*, 1987); M1hc, bovine intestinal 110K (Hoshimaru and Nakanishi, 1987); Dictymhc A, *Dictyostelium* *mhcA* (Warrick *et al.*, 1986); Nematode, *C. elegans unc-54* (Karn *et al.*, 1983); consensus, the most frequently used amino acid at that position; the letter is capitalized if that amino acid is absolutely conserved, and a period indicates that there is no clear consensus at that position. Dots are inserted in the sequences where necessary to maximize the alignment. *, the location of the reactive thiols, SH1 and SH2, one or both of which are typically found in most, but not all, conventional myosins. Note that the *Acanthamoeba* myosin IB gene (Jung *et al.*, 1987) actually encodes the recently described *Acanthamoeba* myosin IC protein and that the *Acanthamoeba* myosin IL gene actually encodes the *Acanthamoeba* myosin IB protein (see footnote in Jung *et al.*, 1989). (B) Restriction map of the *abmA* gene.

I. ATP Binding Region

Dictyabm A -EGENQCIIIS GESGAGKTEA SKYIMQYIAS ITGSSTEVEY VKKT..... ILESNP LLEAFNGKLT LRNNNSSRFG KYMEIQF-
 Dictyabm B -DQENQCVIIS GESGAGKTEA AKLIMGYVSA ISGSTKVEY VKHV..... ILESNP LLEAFNGSKT LRNNNSSRFG KYFEIHF-
 Dictyabm C -EKENQCVIIS GESGAGKTEA AKKIMQYIAD VSGERGSSSN QKVEHVKSI. ILETNP LLEAFGNAKT LRNNNSSRFG KYFEIQF-

 Dictymyo 1 -DQENQCVIIS GESGAGKTEA AKLIMGYVSA ISGSTKVEY VKHV..... ILESNP LLEAFNGSKT LRNNNSSRFG KYFEIQF-
 Acanmyo 1l -ENINQCVIIS GESGAGKTEA SKLVMQYVAA VSGNSGGVDF VKH..... SNP LLEAFNGSKT LRNNNSSRFG KYFEIHF-
 Acanmyo 1b -ESEDQCVIIS GESGAGKTEA SKTEASKKIM QYIAAVSGAT GDVMRVKDV. ILEAFGNAKT LRNNNSSRFG KYMEIQF-
 Mlhc -RDRDQCILIT GESGAGKTEA SKLVMSYVAA VCGKGEQVNS VKEQLLQ... SNP VLEAFGNAKT LRNNNSSRFG KYMDIEF-
 Dictymhc A -DRQNQSLIT GESGAGKTEN TTKVIQYLAS VAGRNQANGS GVLEQQILQANP ILEAFGNAK. LRNNNSSRFG KYFEIQF-
 Nematode -DHENQSMILIT GESGAGKTEN TTKVICYFAA VGASQEGGA EVDPNKKKVT LEDQIVQTNP VLEAFGNAKT LRNNNSSRFG KYFIRIHF-
 consensus edenQcviIs GESGAGKTEa sklvmqyvaa vsgs.eeve. vkhe.vk.v.IlesNP iLEAFGNaKT LRNNNSSRFG KyfeIqF-

II. Actin Binding Region

Dictyabm A -IPTDKKSPIT AGFQFNQVT SLLKSLYCSP HYVRCIKPNS NMRLEWDQS KCAEQVAYLG SFENLLVRRR GYCYRQTFSK-
 Dictyabm B -SLQKKRPTTA GFKIKTSAGE LMKALSQCTP HYIRCIPNE TKKAKDWENS RVKHQVQYLG LLENVRVRR

 Dictymyo 1 -SLQKKRPTTA GFKIKTSAGE LMKALSQCTP HYIRCIPNE TKKAKDWENS RVKHQVQYLG LLENVRVRRR GFAYRNTFDK-
 Acanmyo 1l -QLQKKRPTTA GFKLKTSADA LMEALSRCSP HYIRCIPND NKAYHDWDAT RTKHQVQYLG LLENVRVRRR GFAYRAEFDR-
 Acanmyo 1b -ATSKKKPTTA GFKIKESINI LVATLSKCTP HYIRCIPNE KKAANAFNNS LVLHQVKYLG LLENVRIRRA GYAYRQSYDK-
 Mlhc -QASLKRPTTA GAQFKSSVIT LMKNLYSKNP NYIRCIPNE HQQRGHFSFE LVSVAQYLG LLENVRVRRR GYAYRQAYGS-
 Dictymhc A -RAKKGAFITV AAQYKEQLAS LMATLETNP HFVRCIIPNN KQLPAKLEDK VVLDQLRCNG VLEGIRITRK GFPNRIIYAD-
 Nematode -KGKSGSMTV SMLYRESLNN LMTMLNKTHP HFIRCIPNE KKQSGMIDAA LVLNQLTCNG VLEGIRICRK GFPNRTLHPD-
 consensus slqkkrrptta gfkiktslge lmkalsqctP hyiRCIkPNe kkkagdwens lvlhQvqylg llenvrvrRa GfayRqtfdk

* *
 SH2 SH1

Figure 4. Dot matrix analyses of amino acid sequence homologies between *abmA* and other actin-based motors. (A) *abmA* vs. *Dictyostelium* myosin I (Jung *et al.*, 1989). (B) *abmA* vs. *Acanthamoeba* myosin IL (Jung *et al.*, 1989; the sequence spanning residues 910 to 1096 was unavailable, and a spacer was inserted to preserve spatial relationships). (C) *abmA* vs. *Acanthamoeba* myosin IB (Jung *et al.*, 1987). (D) *abmA* vs. bovine intestinal M1hc (Hoshimaru and Nakanishi, 1987). (E) *abmA* vs. *mhcA* (Warrick *et al.*, 1986). The matrices shown were generated by placing a dot every time 10 residues in a window of 16 exactly matched. The numbers on the axes indicate the position in the deduced amino acid sequence. The small black arrowhead in each plot indicates the deduced position of the S1/S2 junction, an enzymatic cleavage site where chymotrypsin separates rabbit skeletal muscle myosin into S1 and tail (see Warrick and Spudich, 1987). The large open arrow in each plot highlights homologies found in the putative membrane-binding domain of the tail.

stitutions. Neither of the reactive thiols, SH1 and SH2, are present.

Dot-matrix comparisons show that the N-terminal two-thirds of *abmA* is quite homologous to *Dictyostelium* myosin I, *Acanthamoeba* myosins IB and IL, bovine intestinal M1hc and *Dictyostelium mhcA* head regions (Figure 4). Allowing for the addition of limited gaps in amino acid sequence alignments, the head region of the *abmA*

gene is 40% identical to the analogous region of *Dictyostelium mhcA* (Table 1). Under the same conditions *abmA* is 50% identical to either the *Dictyostelium* myosin I, *Acanthamoeba* myosins IB and IL or bovine intestinal M1hc head regions (Table 1). As is observed in other small myosin genes, the *abmA* tail sequence lacks the characteristic hydrophobic repeats that are responsible for the coiled-coil structure of a conventional

Table 1. Myosin head region amino acid sequence homology

Actin-based motors	Amino acid homology (%)				
	DictymhcA	Bovine M1hc	Acanmyo 1L	Acanmyo 1B	Dictymyo 1
Dictyabm A	40	49	51	49	50
Dictymyo 1	39	45	65	55	
Acanmyo 1B	39	47	55		
Acanmyo 1L	40	49			
Bovine M1hc	41				

The head region is defined as the sequence that corresponds by homology to the chymotryptically derived S1 fragment of rabbit skeletal myosin. Homology is defined by identities in pair-wise sequence alignments. The alignments were made with the program BESTFIT (Devereux *et al.*, 1984) allowing limited gaps (gap weight: 3.0, gap length weight: 0.1).

myosin tail. *abmA* shows little homology with the C-terminal tail sequence of M1hc, but some homology with the amino terminal portion of the tails of *Dictyostelium* myosin I and *Acanthamoeba* myosins IB and IL (see large, open arrows in Figure 4, A–C).

Southern analysis of wild type *Dictyostelium* genomic DNA (Figure 1C) demonstrates that the 3.3 kb *abmA* *Bgl* II fragment does not cross-hybridize with expected *mhcA* gene fragments (De Lozanne *et al.*, 1985) under high stringency. A series of faint bands are observed in Figure 1C, indicating that *abmA* may be part of a multigene family. Note that the pattern of hybridization is distinct from that observed in Figure 1A, where the *mhcA* probe was used, confirming the presence of additional genes which may contain sequences similar to the myosin head. Fragments expected from hybridization to the *Dictyostelium* myosin I gene (Jung *et al.*, 1989) are not apparent with either the *mhcA* or the *abmA* probes (Figure 1, A and C).

Additional clones were identified in a *mhcA*[−] genomic EMBL3 *Bcl* I library using the same *mhcA* probe used to find *abmA*. Two genes were isolated and nucleotide sequence data confirmed that they are distinct from the *abmA* gene. We refer to these clones as *abmB* and *abmC*, based on a comparison of the deduced amino acid sequences from portions of these clones with *abmA* and with other known myosin head regions (Figure 3). *abmB* contains both the typical myosin ATP-binding site as well as the actin binding site (Figure 3). The sequence is identical to *Dictyostelium* myosin 1, indicating that they are probably the same gene. Northern analysis using probes from both *abmB* and *abmC* clones reveals that these genes are expressed in *Dictyostelium* and encode for polypeptides of $\leq 135\,000$ Da (data not shown).

Discussion

We have identified a novel actin-based motor gene in *Dictyostelium* (*abmA*). Detailed analysis of the *abmA* sequence reveals that the N-terminal two-thirds of the deduced amino acid sequence is homologous to the head regions of a number of other myosins (compare Figures 2 and 3 to Figure 6 in Warrick and Spudich, 1987). The *abmA* gene encodes for a protein that contains both the highly conserved myosin ATP binding site (Walker *et al.*, 1982) as well as a sequence which is believed to be involved in the binding of the myosin head to actin (Sutoh, 1983). The presence of these two well-defined myosin sequences in the N-terminal portion of this gene makes it likely that

the encoded protein is indeed an actin-based motor.

The results of Northern analysis demonstrate that the *abmA* gene product is ~ 135 kDa, which is considerably smaller than the conventional myosin heavy chain. As is true for the myosin I proteins, the C-terminal tail region of *abmA* lacks both the alpha-helical content, as determined by secondary structure prediction methods (Chou and Fasman, 1978), and the characteristic hydrophobic repeats necessary for forming an alpha-helical coiled-coil typical of a conventional myosin. Strikingly, the unusual Gly,Pro,Ala-rich tail of the C-terminal portion of the *Dictyostelium* and *Acanthamoeba* myosin Is (Jung *et al.*, 1987, 1989), which is in the ATP-insensitive actin binding domain (Lynch *et al.*, 1986), is not present in *abmA*. The bovine intestinal M1hc protein also lacks the Gly,Pro,Ala repeats (Hoshimaru and Nakanishi, 1987).

Biochemical support for the potential role of actin-based motors other than conventional myosin in cellular motility is provided by the ability of *Acanthamoeba* myosin IB (Albanesi *et al.*, 1985) and the brush border 110K-calmodulin complex (Mooseker and Coleman, 1989) to support movement *in vitro*. Additionally, native *Acanthamoeba* vesicles have been shown to move along *Nitella* actin cables and this movement is inhibited by monoclonal antibodies directed against *Acanthamoeba* myosin I (Adams and Pollard, 1986). Both *Acanthamoeba* myosin IA and IB have also been shown to be associated with the plasma membrane (Gadasi and Korn, 1980).

Adams and Pollard (1989) demonstrated that the *Acanthamoeba* myosin I directly binds to *Acanthamoeba* membrane lipids as well as vesicles of pure phosphatidyl serine, and their evidence suggests that the N-terminal half of the myosin I tail may interact with the vesicle surface via charge-charge interactions. Dot-matrix analyses show that the *abmA* tail has an interesting sequence homology with this putative membrane-binding domain of the *Acanthamoeba* myosin I tail and a similar homology is observed in the comparison of *abmA* to the *Dictyostelium* myosin I (Figure 4, A–C). The homologous domain contains short clusters of basic residues separated by longer stretches of uncharged amino acids (Figure 5). This conserved domain may play a role in the attachment of these actin-based motors to plasma membranes or vesicles.

The gene encoding the previously described *Dictyostelium* myosin I (Cote *et al.*, 1985; Lynch *et al.*, 1988) has been sequenced (Jung *et al.*, 1989). Based on low-stringency Southern analysis using a portion of the gene encoding the divergent tail region of myosin I, Jung *et al.* (1989) conclude

```

Dictyabm A  VSRHEKIQKRSLLVTNETIF.....TITPHKPKDGSWF.AIKRKVPFSAIEKISFSKLSDDFFVHIINEHDLCLETNKTVLITLLSNLYSKHLNGKELVFEFKDS
Dictyabm 1  IDRRAKQKNYEMVLTDQALY.....FVEKSIKKVLVH..TLIRRVGLREIKGVSISLSDNVIVFH.LPEHDQVIENDKKTEIIIVLVEYF.KAIGGSLNVQFSDR
Acanmyo 1L  LNRRGKPERRDLIVTNEAVY.....FAMRKKSGQVVYNLKRRIPLGEIASLSSLTLQDNVVVIH.HNQYDMVFENDKKTEIIVTLMENY.KMSGGRDLPVFNDN
Acanmyo 1B  SPWGSKVERRIMVLTSNAMFLVAIDPNKDKIEKVKPFLYVLKRRIDFNKIGSITLSPLQDNFMLISVNGEHSNLLECRRKTELIGVLLKNPSVRIQFADTFNVTLK
Basic      * * * * *          * * *          * * *          * * *          * * *          *
Consensus  ..rr.K.err...vTnea.f.....f.p.ikKKvg...y.lkRrvpf.eI.sislStLsDnf.vih..nehd.vlEndkKTELitvL.e.y.k..gg..l.vnf.d.

```

Figure 5. Amino acid sequences of the putative membrane-binding domain of the *abmA* and myosin I tails. Dictyabm 1, *Dictyostelium* myosin I (Jung *et al.*, 1989); Acanmyo 1B, *Acanthamoeba* myosin IB (Jung *et al.*, 1987); Acanmyo 1L, *Acanthamoeba* myosin IL (Jung *et al.*, 1989); consensus, the most frequently used amino acid at that position; the letter is capitalized if that amino acid is absolutely conserved, and a period indicates that there is no clear consensus at that position. Dots are inserted in the sequences where necessary to maximize the alignment. The bold letters indicate basic residues (highlighted by *). The sequences shown, from top to bottom, begin at amino acid 785, 801, 762, and 759, respectively.

that there is a single *Dictyostelium* myosin I gene and that myosin I and the conventional myosin might be the only actin-based motors present in *Dictyostelium*. To identify all possible actin-based motors, however, the probe of choice is one that includes all of the highly conserved regions of the myosin head. We have used such a probe to screen genomic libraries generated from *mhcA*⁻ DNA to identify all possible genes containing conserved myosin sequences. The *abm* genes isolated using this approach and described here are a family of small myosins which include *Dictyostelium* myosin I.

That *Dictyostelium* contains multiple genes encoding for actin-based motor proteins is not surprising. *Acanthamoeba* has been shown to have at least three distinct myosins (Horowitz and Hammer, 1988; Korn and Hammer, 1988). The identification of genes encoding multiple actin-based motors in *Dictyostelium*, an organism that is amenable to molecular genetic manipulation, provides an opportunity to study the role of these various motors in vivo. These molecules are of great interest since recent experiments have demonstrated that the conventional myosin is not required for the movement of a cell across a surface, the extension and retraction of filopodia, pseudopodia, or lamellipodia, or the movement of intracellular particles (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Peters *et al.*, 1988; Wessels *et al.*, 1988; Manstein *et al.*, 1989).

Methods

Genomic DNA from *Dictyostelium* was isolated from *mhcA*⁻ cells (line A5-2) as described in Manstein *et al.* (1989). Southern blotting was performed as described by Howley *et al.* (1979). Several probes were used in the Southern blotting. A 1.8 kb 5' fragment derived from the *Dictyostelium* conventional myosin heavy chain gene (*mhcA*) was used to screen both Southern blots and genomic libraries. This *mhcA* probe spans from the ATG start codon to the first *Bgl* II site which lies within the myosin head, immediately preceding the thiol region (Warrick *et al.*, 1986). Southern blots were also hybridized with probes derived from the myosin head region of genes from a variety of sources: a 1.4 kb *Bgl* II fragment derived from a *Bam*HI/*Xba*I subclone of the *Acanthamoeba* myosin IB gene (Jung *et al.*, 1987); a 5' 1.9 kb *Xba*I/*Bam*HI fragment from the

Acanthamoeba lambda 3.5 gene (Horowitz and Hammer, 1988); a 2 kb *Eco*RI/*Pst* I fragment from *Drosophila* *ninaC* clone pc-ninaC-15R (Montell and Rubin, 1987); and a 5' 2.7 kb *Bam*HI fragment from the *C. elegans* *unc-54* lambda clone SG23-10 (MacLeod *et al.*, 1981). Hybridization of *mhcA*⁻ DNA was performed in 40% formamide and 6× SSC at 37°C and the blots washed in 6× SSC at 65°C. The hybridization of *abmA* to wild type DNA was performed in 50% formamide and 6× SSC and the blots washed in 2× SSC at 72°C (1× SSC consists of 0.015 M Na·citrate, 0.15 M NaCl).

The construction and screening of genomic libraries and all other cloning procedures were as described in Maniatis (1980). All enzymes used, unless otherwise stated, were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Two genomic libraries were constructed, a *Bcl* I library and a *Bgl* II library, in EMBL3 (Stratagene, La Jolla, CA). The libraries were screened using the *Dictyostelium* *mhcA* fragment as a probe. The genomic fragments were subcloned into pTZ-18R (Pharmacia LKB Biotechnology, Piscataway, NJ). Nested deletions were constructed using the Erase-A-Base system (Promega, Madison, WI) and sequencing performed using the Sequenase system (US Biochemicals, Cleveland, OH).

DNA sequences obtained were analyzed using the UWGCG Sequence Analysis Software Package (version 6) developed by Devereux *et al.* (1984).

Acknowledgments

We thank John Tan for his patience and helpful suggestions throughout the course of this project, Jeff Finer for helping with the genomic screening, and Shoshana Ravid for carefully reading the manuscript. We also thank Drs. John Hammer, Jill Horowitz, Goeh Jung, Jonathan Karn, and Craig Montell for their gifts of plasmids containing myosin gene sequences used as probes in the early experiments. This work was supported by an NIH postdoctoral fellowship (GM-12000) to M. A. Titus and an NIH grant (GM-40509) to J. A. Spudich.

Received: September 5, 1989.

References

- Adams, R.J., and Pollard, T.D. (1986). Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin-I. *Nature* 322, 754–756.
- Adams, R.J., and Pollard, T.D. (1989). Binding of myosin I to membrane lipids. *Nature* 340, 565–568.
- Albanesi, J.P., Fujisaki, H., Hammer, J.A., III, Korn, E.D., Jones, R., and Sheetz, M.P. (1985). Monomeric *Acanthamoeba* myosins I support movement in vitro. *J. Biol. Chem.* 260, 8649–8652.

- Chou, P.Y., and Fasman, G.D. (1978). Predictions of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol.* **47**, 797–822.
- Collins, J.H., and Borysenko, C.W. (1984). The 110,000-dalton actin- and calmodulin-binding protein from intestinal brush border is a myosin-like ATPase. *J. Biol. Chem.* **259**, 14128–14135.
- Clarke, M., and Spudich, J.A. (1974). Biochemical and structural studies of actomyosin-like proteins from non-muscle cells. Isolation and characterization of myosin from amoebae of *Dictyostelium discoideum*. *J. Mol. Biol.* **86**, 209–222.
- Cote, G.P., Albanesi, J.P., Ueno, T., Hammer, J.A., III, and Korn, E.D. (1985). Purification from *Dictyostelium discoideum* of a low-molecular weight myosin that resembles myosin I from *Acanthamoeba castellanii*. *J. Biol. Chem.* **260**, 4543–4546.
- De Lozanne, A., Lewis, M., Spudich, J.A., and Leinwand, L.A. (1985). Cloning and characterization of a nonmuscle myosin heavy chain cDNA. *Proc. Natl. Acad. Sci. USA* **82**, 6807–6810.
- De Lozanne, A., and Spudich, J.A. (1987). Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science* **236**, 1086–1091.
- Devereux, J., Haeberti, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the Vax. *Nucleic Acids Res.* **12**, 387–395.
- Gadasi, H., and Korn, E.D. (1980). Evidence for differential intracellular localization of the *Acanthamoeba* myosin isoenzymes. *Nature* **286**, 452–456.
- Horowitz, J.A., and Hammer, J.A., III. (1988). Characterization of a gene for a myosin-like protein. *Biophys. J.* **53**, 581a.
- Hoshimaru, M., and Nakanishi, S. (1987). Identification of a new type of mammalian myosin heavy chain by molecular cloning. Overlap of its mRNA with preprotachykinin B mRNA. *J. Biol. Chem.* **262**, 14625–14632.
- Howley, P.M., Israel, M.A., Law, M.F., and Martin, M.A. (1979). A rapid method for detecting and mapping homology between heterologous DNAs. Evaluation of polyomavirus genomes. *J. Biol. Chem.* **254**, 4876–4883.
- Jung, G., Korn, E.D., and Hammer, J.A., III. (1987). The heavy chain of *Acanthamoeba* myosin IB is a fusion of myosin-like and non-myosin-like sequences. *Proc. Natl. Acad. Sci. USA* **84**, 6720–6724.
- Jung, G., Saxe, C.L., III, Kimmel, A.R., and Hammer, J.A., III (1989). *Dictyostelium discoideum* contains a gene encoding a myosin I heavy chain. *Proc. Natl. Acad. Sci. USA* **86**, 6186–6190.
- Karn, J., Brenner, S., and Barnett, L. (1983). Protein structural domains in the *Caenorhabditis elegans* unc-54 myosin heavy chain gene are not separated by introns. *Proc. Natl. Acad. Sci. USA* **80**, 4253–4257.
- Korn, E.D., and Hammer, J.A. III. (1988). Myosins of nonmuscle cells. *Annu. Rev. Biophys. Chem.* **17**, 23–45.
- Knecht, D.A., and Loomis, W.F. (1987). Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. *Science* **236**, 1081–1086.
- Lynch, T.J., Albanesi, J.P., Korn, E.D., Robinson, E.A., Bowers, B., and Fujisaki, H. (1986). ATPase activities and actin binding properties of subfragments of *Acanthamoeba* myosin IA. *J. Biol. Chem.* **261**, 17156–17162.
- Lynch, T.J., Brzeska, H., Lambooy, P.K., and Korn, E.D. (1988). Physical and enzymatic properties of *Dictyostelium* myosin I. *J. Cell Biol.* **107**, 7a.
- MacLeod, A.R., Karn, J., and Brenner, S. (1981). Molecular analysis of the unc-54 myosin heavy-chain of *Caenorhabditis elegans*. *Nature* **291**, 386–390.
- Maniatis, T., Fritsch, E.F., and Sambrook, J. (1980). Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Manstein, D.J., Titus, M.A., De Lozanne, A., and Spudich, J.A. (1989). Gene replacement in *Dictyostelium*: generation of myosin null mutants. *EMBO J.* **8**, 923–932.
- Montell, C., and Rubin, G.M. (1988). The *Drosophila* ninaC locus encodes two photoreceptor cell specific proteins with domains homologous to protein kinases and the myosin heavy chain head. *Cell* **52**, 757–772.
- Mooseker, M.S., and Coleman, T.R. (1989). The 110-kD protein-calmodulin complex of the intestinal microvillus (brush border myosin I) is a mechanoenzyme. *J. Cell Biol.* **108**, 2395–2400.
- Mooseker, M.S. (1985). Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. *Annu. Rev. Cell Biol.* **1**, 209–241.
- Peters, D.J.M., Knecht, D.A., Loomis, W.F., De Lozanne, A., Spudich, J.A., and Van Haastert, P.J.M. (1988). Signal transduction, chemotaxis, and cell aggregation in *Dictyostelium discoideum* cells without myosin heavy chain. *Dev. Biol.* **128**, 158–163.
- Pollard, T.D. (1981). Cytoplasmic contractile proteins. *J. Cell Biol.* **91**, 156s–165s.
- Pollard, T.D., and Korn, E.D. (1973). *Acanthamoeba* myosin. I. Isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. *J. Biol. Chem.* **248**, 4682–4690.
- Sutoh, K. (1983). Mapping of actin-binding sites on the heavy chain of myosin subfragment-1. *Biochemistry* **22**, 1579–1585.
- Titus, M.A., Warrick, H.M., and Spudich, J.A. (1988). Identification of a non-conventional myosin gene in *Dictyostelium* by heterologous probes. *J. Cell Biol.* **107**, 471a.
- Toyoshima, Y.Y., Kron, S.J., McNally, E.M., Niebling, K.R., Toyoshima, C., and Spudich, J.A. (1987). Myosin subfragment-1 is sufficient to move actin filaments in vitro. *Nature* **328**, 536–539.
- Walker, J.E., Saraste, M., Runswick, M.J., and Gay, N.J. (1982). Distantly related sequences in the α - and β -subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951.
- Warrick, H.M., De Lozanne, A., Leinwand, L.A., and Spudich, J.A. (1986). Conserved protein domains in a myosin heavy chain gene from *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA* **83**, 9433–9437.
- Warrick, H.M., and Spudich, J.A. (1987). Myosin structure and function in cell motility. *Annu. Rev. Cell Biol.* **3**, 379–421.
- Wessels, D., Soll, D.R., Knecht, D.A., Loomis, W.F., De Lozanne, A., and Spudich, J.A. (1988). Cell motility and chemotaxis in *Dictyostelium* amebae lacking myosin heavy chain. *Dev. Biol.* **128**, 164–177.