Substitution Mutations in the Myosin Essential Light Chain Lead to Reduced Actin-activated ATPase Activity Despite Stoichiometric Binding to the Heavy Chain*

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Myosin essential light chain (ELC) wraps around an α-helix that extends from the myosin head, where it is believed to play a structural support role. To identify other role(s) of the ELC in myosin function, we have used an alanine scanning mutagenesis approach to convert charged residues in loops I, II, III, and helix G of the Dictyostelium ELC into uncharged alanines. Dictyostelium was used as a host system to study the phenotypic and biochemical consequences associated with the mutations. The ELC carrying loop mutations bound with normal stoichiometry to the myosin heavy chain when expressed in ELC-minus cells. When expressed in wild type cells these mutants competed efficiently with the endogenous ELC for binding, suggesting that the affinity of their interaction with the heavy chain is comparable to that of wild type. However, despite apparently normal association of ELC the cells still exhibited a reduced efficiency to undergo cytokinesis in suspension. Myosin purified from these cells exhibited 4–5-fold reduction in actin-activated ATPase activity and a decrease in motor function as assessed by an in vitro motility assay. These results suggest that the ELC contributes to myosin's enzymatic activity in addition to providing structural support for the α-helical neck region of myosin heavy chain.

Myosin is a mechanochemical enzyme that converts the energy of ATP hydrolysis into diverse actin-based cellular movements such as muscle contraction and cytokinesis (1, 2). Conventional myosin is composed of two heavy chains (~200 kDa) and two pairs of light chains (16–20 kDa), the regulatory light chain (RLC)1 and the essential light chain (ELC). Observed in wild type cells these mutants competed efficiently with the endogenous ELC for binding, suggesting that the affinity of their interaction with the heavy chain is comparable to that of wild type. However, despite apparently normal association of ELC the cells still exhibited a reduced efficiency to undergo cytokinesis in suspension. Myosin purified from these cells exhibited 4–5-fold reduction in actin-activated ATPase activity and a decrease in motor function as assessed by an in vitro motility assay. These results suggest that the ELC contributes to myosin's enzymatic activity in addition to providing structural support for the α-helical neck region of myosin heavy chain.

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‡ The abbreviations used are: RLC, regulatory light chain; ELC, essential light chain; MHC, myosin heavy chain; PIPES, 1,4-piperazinediethanesulfonic acid; BSA, bovine serum albumin; DAPI, 4',6-diamidino-2-phenylindole-2HCl; PCR, polymerase chain reaction.
clusters of charged amino acid residues (i.e. two or more charged residues present in a stretch of five) are likely to occupy exposed positions, and substitution of these residues with alanines is structurally conservative at any residues that are not buried deep in the hydrophobic core and hence unlikely to disrupt the overall conformation of the molecule.

The ELC belongs to calmodulin and troponin C gene family and is composed of four helix-loop-helix motifs (26). Of the 14 charged clusters identified in the Dictyostelium ELC sequence, three fall in loop structures, and the others are in helices. To obtain the mutants that are able to stabilize the myosin neck, the surface residues of ELC which do not participate in the binding to the heavy chain were targeted for mutagenesis. The structure of myosin S1 (7) and scallop regulatory domain (8) shows the loops of the ELC to be solvent exposed. Thus the three charged clusters in loops (I, II, III) of the ELC were targeted for mutagenesis. In addition, a charged cluster in the G helix which is adjacent to the fourth loop was mutagenized.

When expressed in Dictyostelium ELC-minus cells, the ELC carrying mutations in loops I, II, III bind to the heavy chain with normal stoichiometry. However, myosin carrying the ELC mutants exhibited decreased actin-activated ATPase activity with normal stoichiometry. Howewer, myosin carrying the ELC sequence, 3', and the tagged ELCs copartitioned (Fig. 3). The pairs of primers (5', 3') used to introduce mutations include (all sequences are shown 5'-3'; mutated sequences are underlined): L1, TTGCGACGCAAATGCAGCCGCTGGAATGCAT; mutated sequences are underlined, GCAGGC- TTGTGTGCGAAAGTGAATGAAACAT; L2, GAGCGATTTGCGATCATAG- TACATTAAAGAG and TGCAAATGCTGCAGCATTAATTCT; L3, GCAGCGGCGCGCGTGTGAGCGCAAGCAGCAGA and CTGGAAGTGGCGG CCGTTGAGATCA; L4, CCGGCTTGATGCGAGTTATFAGAAGAAATGC and TGCGTGAACGTGACGGGTGTGATAAGTAA.

The primer used to introduce the epitope tag (underlined) to the carboxyl terminus of the laminin 1, L2, and L3 cDNAs is 5'-CCGATCTCTTGATTTCAGCA- CCTGCTGCGAAAGCCTTAATGATG-3' with a BamHI site and 2 extra bases included at the 5' end for cloning purposes. All polymerase chain reaction products were sequenced to confirm the occurrence of the desired mutations and the absence of random polymerase chain reaction-induced mutations; they were subsequently cloned into the integrating expression vector pBORG (30) at the BamHI site. Transformation and Growth Conditions—Expression constructs were introduced into Dictyostelium AX3 cells or ELC-minus cells by electroporation (31). Transformants were selected and grown in HL-5 medium containing 10 μg/ml G418 and 100 μg/ml streptomycin.

Preparation of Cell Extracts—Dictyostelium whole cell lysates were prepared as described by Pollenz et al. (15). Myosin extracted from cytoskeletons was prepared according to Giffard et al. (32). Briefly, 1 × 10^7 cells were collected, washed in wash buffer (100 mM PIPES, pH 6.8, 2.5 mM EGTA, 1 mM MgCl_2, 10 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 20 mM benzamidine), and lysed in wash buffer containing 0.25% Triton X-100. The Triton-insoluble pellet (cytoskeletons) was collected and resuspended in wash buffer containing 200 mM KCl and 2 mM ATP. Following centrifugation in a microcentrifuge, the supernatant was recovered, and one-fifth of it was used for analysis.

Western Blot Analysis—Protein samples were electrophoresed on a 15% SDS-polyacrylamide gel, transferred to nitrocellulose, and blotted with myosin polyclonal antiserum to the carboxyl terminus of myosin (29). Antbody binding was detected using the Western blot Chemiluminescence Reagent (Du Pont NEN). For quantification of stoichiometry of myosin subunits, Western blots of myosin extracts from approximately 2 × 10^6 cells were scanned using a laser densitometer (LKB).

Protein Purification—Wild type (JH10) and mutant myosins were purified according to the method described by Uyeda and Spudich (17) and Ruppel et al. (33), except cells expressing mutants were seeded from 40 confluent 24-cm plastic dishes into suspension (10 liters) and grown for approximately 40 h before harvesting for purification. Purified myosin was treated with a recombinant Dictyostelium myosin light chain kinase (a gift of Drs. J. Smith and J. Spudich, Stanford University) as described by Uyeda and Spudich (17). Myosin activity was assayed within 2 days of purification. Stoichiometry of myosin subunits was also determined by separating the heavy and light chains on 5–20% SDS-polyacrylamide gradient gels and staining the protein with Coomassie Brilliant Blue. Gels were scanned using a Bio-Rad densitometer and the Molecular Analyst software (Bio-Rad).

ATPase Assays—Actin-activated ATPase was assayed in 10 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM MgCl_2, 0.1 mM CaCl_2, 1 mM ATP, varying concentrations of cctin (0–10 μM), and 50 μg/ml myosin. ATPase was assayed in 20 mM Tris-HCl, pH 8.0, 500 mM KCl, 10 mM CaCl_2, 1 mM ATP, and 50 μg/ml myosin. Reactions were incubated at room temperature for 5 min, quenched with acid, and the liberated Pi was quantified following organic extraction (34, 35).

Measurement of Myosin Active Heads—The number of myosin active heads was measured using a protocol suggested by Dr. J. R. Sellers (National Institutes of Health). 5 μg of purified myosin (containing 20 pmol of heads) was incubated with 40 pmol of [γ-32P]ATP in 100 μl of actin-ATPase assay buffer (no actin) as above. Following a 20-s incubation at room temperature, 1 μl unlabeled ATP was added to chase the hydrolysis of the labeled ATP. The liberated Pi was extracted after 10 min by the organic partition method as above.

In Vitro Motility Assays—In vitro motility assays were performed as described previously (3, 36–38). Myosin was diluted to 200 μg/ml in buffer AB (10 mM imidazol, 1 mM ATP, 1 mM EGTA, 10 mM dithiothreitol), applied to a flow cell coated with nitrocellulose, and blocked with AB containing 0.5 mg/ml BSA (AB/BSA). To remove myosin heads that bound actin in a rigor fashion, a solution of phallolidin-labeled actin was perfused following 1 mM ATP in AB/BSA. After washing with AB/BSA to remove the excess nonfluorescent actin, a solution of rhodamine-phalloidin-labeled actin in AB/BSA was introduced. Movement was initiated at room temperature by introducing AB/BSA containing 1 μl ATP and oxygen scavenger enzymes.

DAPI Staining—DAPI staining was performed as described previously (15).

RESULTS
Association of the ELC Mutants with MHC—Charged residues in loops I, II, III, and helix G of Dictyostelium ELC were mutated by the polymerase chain reaction-based mutagenesis approach. Three or four charged amino acid residues in each mutant were replaced with alanines (Fig. 1). The approximate locations of these charged residues are illustrated on the three-dimensional structure of chicken skeletal muscle myosin S1 head (Fig. 1). When expressed in Dictyostelium ELC-minus cells using the expression vector pBORG (30), the mutant ELCs accumulated to levels similar to the endogenous ELC in wild type cells (Fig. 2A). All mutants bound to the heavy chain as indicated by their ability to copartition with the MHC in ATP-extracted cytoskeletal preparations (Fig. 2A). Based on densitometric analysis of Western blots of these preparations, as well as direct staining of purified protein with Coomassie Blue (Fig. 2B), the loop mutants L1, L2, and L3 associated with the heavy chain with 1:1 molar ratio, whereas the helix mutant L4 bound at 0.2 mol/mol heavy chain (Table I).

ELC Loop Mutants Have Normal Affinities for the MHC in Vivo—To assess the binding affinity of mutants L1, L2, and L3, they were tagged at the carboxyl terminus by the addition of five amino acid residues (EQKLL) of a myc epitope, which does not affect the ELC-MHC association or myosin function (29). The tagged mutants were expressed in wild type cells using the pBORG vector. A Western blot of the respective cell lysates shows the expression of the mutants that have a larger size than the endogenous ELC (Fig. 3A). Analysis of supernatants from ATP-extracted cytoskeletons indicates that the endogenous and the tagged ELCs copartitioned (Fig. 3B), indicating that both species associated with the MHC. The relative ex-
pression levels of the tagged versus endogenous ELCs were 1:1 in cell lysates, and similar ratios were obtained from purified myosin, suggesting that the mutant ELCs have an affinity for the heavy chain similar to that of wild type.

**ELC Charge Mutants Have Defective Actin-activated ATPase Activities**—Myosin was purified from both wild type and ELC mutant cell lines by a rapid purification method developed by Uyeda and Spudich (17) and Ruppel et al. (33). This method involves three rounds of salt-dependent assembly and disassembly of myosin filaments. Although we could not purify myosin from ELC-minus cells by this method, myosins carrying the ELC charge mutations were recovered as efficiently as wild type, indicating that they possessed assembly and disassembly properties typical of wild type myosin. The normal 1:1 stoichiometry of the three myosin subunits was maintained after purification (Fig. 2B).

When assayed for actin-activated ATPase, myosins with L1, L2, and L3 ELCs exhibited a 4–5-fold reduction in the activity compared with wild type (Table II). In contrast, the high salt Ca$$^{2+}$$-ATPase, a property that assesses the integrity of the active site, was within the normal range for all three mutants (Table II), suggesting that each of the mutant myosins was capable of nucleotide hydrolysis. Because the actin-activated ATPase was greatly reduced in mutants L1, L2, and L3, it was important to know whether this was due to a significantly increased number of inactive molecules in the mutant myosin preparations. A crude assay was employed to determine the relative number of active heads in each myosin preparation. This assay was performed by incubating myosin with labeled ATP at 1:2 molar concentrations for a time duration short enough to allow each head the chance to hydrolyze a single labeled ATP. The amount of radioactive phosphate liberated therefore provides a crude assessment of the relative number of active heads. As shown in Table II, myosin preparation containing ELC mutants L1, L2, and L3 showed 81, 66, and 118% of the number of active heads of wild type preparations. Thus it is unlikely that the reduced actin-activated ATPase is due to a decreased stability of the mutant myosins but rather is the result of a decreased turnover rate of the enzyme.

Like other non-muscle myosins, the actin-activated ATPase of *Dictyostelium* myosin is regulated by RLC phosphorylation (39). Myosin purified by the rapid method has a low level of the phosphorylated RLC (33). When these myosins were treated with a recombinant myosin light chain kinase (40), the actin-activated ATPase activity of wild type was increased to 163 nmol/min/mg, representing an approximately 3-fold enhancement (Table II and Fig. 4). The activity of the mutants also increased 3–4-fold after phosphorylation (Table I), but the $V_{max}$ was reduced greatly relative to wild type (Fig. 4). The kinase treatment resulted in uniformly high levels of RLC phosphorylation in both wild type and mutant myosins (data not shown). Analysis of the $K_m$ for actin on kinase-treated myosins yielded similar values between the mutants and wild type (Table II), suggesting that the reduced actin-activated ATPase activity is not due to a decreased affinity for actin. When examined for low salt Mg$$^{2+}$$-ATPase, the wild type and mutant myosins exhibited similar basal activities of 6–8 nmol/mg/min.

There is a good correlation between the actin-activated ATPase activity and the motor function of myosin as assayed by *in vitro* motility assays (1). But uncoupling of the two events has been observed in several recent studies (13, 17, 41). When analyzed by an *in vitro* motility assay, myosins containing ELC charge mutations moved actin filaments at rates of 0.58–0.84 μm/s compared with 2.1 μm/s for wild type (Fig. 5). The mutant myosins did not generate significant actin movement without prior treatment with myosin light chain kinase.

**Dictyostelium Cells Expressing the Mutant ELCs Perform Cytokinesis with Reduced Efficiency**—Myosin is essential for cytokinesis of *Dictyostelium* when grown in suspension (14–16, 42–44). When cells expressing the mutants L1, L2, and L3 were analyzed for their ability to perform cytokinesis in suspension, many cells with multiple nuclei were observed (Fig. 6). The cytokinesis defect was quantified further by scoring the percentage of multinucleate cells (with three or more nuclei) in

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**Figure 1.** Mutated charged residues in the ELC and their spatial locations on the S1 head. Shown in the table are locations of the charged residues (underlined) that were replaced with alamines in each ELC mutant. Locations of these charged residues on the crystal structure of S1 head (7) are determined by sequence alignment with the skeletal muscle ELC.

| Mutants | Sites of Mutation | Substituted Residues |
|---------|------------------|----------------------|
| L1      | Loop 1           | 16 AspLysAspAsnAsp 20 |
| L2      | Loop 2           | 54 LysGluPheAsp 57   |
| L3      | Loop 3           | 58 AspLysGlu 90      |
| L4      | Helix G          | 115 GluValAspGlu 118 |

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**Figure 2.** Expression and association of the ELC charge mutants in ELC-minus cells. Panel A, cell lysates of 5 × 10$$^5$$ cells from respective transformants (left) and myosins extracted from Triton-insoluble cytoskeletons of the corresponding transformants (right) were subjected to Western blot analysis. The blots were probed with a myosin polyclonal antibody NU48. wt, wild type. Panel B, myosin was purified from cells expressing wild type, L1, L2, and L3 ELCs and separated on a 5–20% SDS-polyacrylamide gel that was then stained with Coomassie Brilliant Blue.
each cell population. As shown in Fig. 7, cells carrying L1, L2, and L3 mutants contained 30, 40, and 28% multinucleated cells, in contrast to 5–6% observed in ELC-minus cells expressing a wild type copy ELC or parental JH10 cells. Furthermore, the phenotype worsened progressively, and by day 6 the multinucleate population rose to 50–60% the majority of which contained six or more nuclei. Those big cells eventually lysed. Thus it is clear that the L1, L2, and L3 mutants show decreased myosin function in vivo.

**DISCUSSION**

It has been hypothesized that myosin essential light chains play a structural support role for the neck of myosin, perhaps by providing added rigidity for the proposed lever arm function of the neck (7, 8). This concept is reinforced by the atomic resolution structures showing that the ELC envelopes a naked α-helix that forms the backbone of the neck domain (7, 8), as well as biochemical studies that show a linear relationship between the length of the light chain binding domain and the rate with which actin filaments are translocated using in vitro motility assays (18). In an attempt to explore other possible role(s) of ELC, a site-directed mutagenesis approach was taken to identify the mutants that bind to heavy chain, and therefore maintain the stability of the neck, but which otherwise impair myosin function. The results showed that substitution mutations of the charged residues in loops I, II, and III of Dictyostelium ELC did not affect the ability of the ELC to bind the heavy chain. However, myosin carrying these mutations exhibited a 4–5-fold reduction in actin-activated ATPase activity which was coupled to a 2.5–3.6-fold reduction in the velocity of actin filament translocation in vitro. These defects in myosin produced Dictyostelium cells with reduced cytokinesis efficiency.

Assessment of the affinity of mutant ELCs for the heavy chain using in vivo competition showed that mutant ELCs compete efficiently with the endogenous wild type ELC, suggesting that the MHC binding affinities are similar. The depressed actin-activated ATPase activity therefore seems unlikely to be caused by gross structural changes or inappropriate binding of the ELC to the α-helix of MHC. Instead the defects observed most likely result from local changes in charge distribution or side chain orientation in the ELC mutants. Because myosin carrying the mutant light chains could be purified through rounds of filament assembly and disassembly, the charge-to-alanine substitutions do not affect myosin filament formation significantly. Furthermore, the stability of purified mutant myosins, measured as the number of active heads relative to the wild type, showed levels of 81, 66, and 118%. The slightly reduced number of active heads observed in the L1 and L2 myosin preparations is not sufficient to produce the 4–5-fold reduction observed in the actin-activated ATPase. Similar to wild type, the loop mutants showed a 3–4-fold enhancement of activity following myosin light chain kinase treatment, indicating that the mutations did not alter the regulation associated with the RLC phosphorylation but instead lowered the basal rate of actin-activated ATP hydrolysis. Because the mutant myosins had a $K_m$ for actin similar to that of wild type, the reduced basal rate of ATP hydrolysis was not due to a change in the affinity for actin.

**What mechanism might account for the depressed actin-activated ATPase seen with the ELC charge mutations? The observed actin-activated ATPase seen with the ELC charge mutations**

**TABLE I**

| Myosin         | Antibody staining of ATP-extracted cytoskeletons$^a$ | Coomassie staining of purified myosin$^b$ |
|----------------|---------------------------------------------------|------------------------------------------|
| Wild type      | 1.0                                               | 1.00 $\pm$ 0.12                          |
| L1             | 1.16                                              | 1.01 $\pm$ 0.16                          |
| L2             | 0.93                                              | 1.23 $\pm$ 0.21                          |
| L3             | 0.83                                              | 0.98 $\pm$ 0.35                          |
| L4             | 0.25                                              | Not determined                           |

$^a$ ATP extracts of Triton cytoskeletons were blotted to nitrocellulose and probed with NU48, a polyclonal antibody that recognizes MHC, RLC, and ELC. Values presented are averages of two independent determinations.

$^b$ Purified myosin was run on 5–20% SDS-polyacrylamide gels and stained with Coomassie Brilliant Blue (for an example, see Fig. 2B). Values presented are the means and standard deviations of four independent determinations.

**FIG. 3. The tagged ELC mutants and the endogenous ELC copartition in myosin cytoskeletal extracts.** Cell lysates of wild type cells transformed with the tagged ELC mutants (panel A) and corresponding myosin extracts from respective cytoskeletons (panel B) were subjected to Western blot analysis. Panel A was probed with NU48 to show the relative positions of the RLC, endogenous ELC, and the tagged ELCs. Panel B was probed with an ELC monoclonal antibody 10A2 to show the coexistence of both mutants and endogenous ELC in the myosin extracts (polyclonal antibody NU48 gave similar results).

**TABLE II**

| Actin-ATPase$^b,a$ | $Ca^{2+}$-ATPase$^b$ | Active heads$^{c,d}$ | Activation ratio$^b$ by MLCK | $K_m$ (actin)$^a$ |
|--------------------|---------------------|----------------------|-----------------------------|------------------|
| $\mu$mol/min/mg     | $\mu$mol/min/mg     | %                    |                             | $\mu$m           |
| Wild type           | 52 $\pm$ 4 (3)      | 410 $\pm$ 70         | 100                         | 3.1              |
| L1                  | 10 $\pm$ 7 (4)      | 330 $\pm$ 47         | 81                          | 4.2              |
| L2                  | 10 $\pm$ 6 (4)      | 315 $\pm$ 35         | 66                          | 2.9              |
| L3                  | 13 $\pm$ 8 (3)      | 900 $\pm$ 200        | 118                         | 3.4              |

$^a$ Values were obtained before kinase treatment.

$^b$ The actin-ATPase of myosin was measured in the presence of 10 μM actin. Numbers in parentheses are the number of independent protein preparations.

$^c$ The number of myosin active heads was assessed from the amount of radioactive phosphate liberated by a single ATP turnover under conditions described under “Materials and Methods.” The numbers are relative to the purified wild type myosin.

$^d$ The activation ratio was derived from quotient of the actin-activated ATPase activities of myosin after and before the myosin light chain kinase (MLCK) treatment.

$^e$ $K_m$ values were determined by a double-reciprocal plot of the data in Fig. 4.
atomic structure of myosin S1 head suggests that the ELC does not contribute directly to formation of the active site. However, based on the chicken S1 structure, the ELC has a substantial protein-protein interface with amino acid residues 720–730 of the heavy chain (7, 45). In addition, the recently reported structures of the Dictyostelium motor domain (46, 47) suggest that the COOH terminus exists in dramatically different positions in MgADP\_BeFx and MgADP\_AlF4 structures thought to mimic the ATP bound and transition state for hydrolysis, respectively. This arrangement would allow movements of this domain to be transduced to the ELC which may in turn modulate or amplify the conformational changes in the active site during the binding and release of nucleotides (7, 48), thereby facilitating the ATPase cycle. Thus finding that mutations in the ELC reduce the basal rate of actin-stimulated ATP hydrolysis supports the idea that the ELC could in some way modulate the communication between the active site and other domains of the head such as the actin binding face. Since the ELC has not been shown to contain known regulatory elements, it is plausible that the MHC head together with the ELC determine the basal activity of the actin ATPase. Consistent with this idea, removal of the neck domain (which binds ELC) produced a motor domain that exhibits higher actin-activated ATPase activity than does intact myosin (49, 50).

Fisher et al. (46) have suggested that the ELC may be important for stabilizing the COOH-terminal regions of the motor domain and perhaps in transmitting the conformational changes induced by ATP hydrolysis. The results presented here provide direct experimental support for this idea. It is interesting to note that the mutations in L3 are located along an interface between the ELC which has also been shown to contain several \(\beta\)-MHC mutations that have been implicated in familial hypertrophic cardiomyopathy (51). This result raises the possibility that mutations in the ventricular ELC (vMLC1) might also lead to cardiac defects. Mutations of both the ELC and RLC have recently been shown to produce hypertrophic cardiomyopathy (52). One of the ELC mutations identified in this study is located at a position very near the L3 mutation described here. Based on the S1 structure, the L3 mutation, which is in the COOH-terminal lobe of the ELC, directly interfaces with the NH\(_2\)-terminal domain of the heavy chain; whereas the L1 and L2 mutations, which are in the NH\(_2\)-terminal lobe of the ELC, do not seem to contact that domain (Fig. 1 and Ref. 7). Nevertheless, a similar degree of reduction in the actin-activated ATPase activity in all three mutations was observed, suggesting that either there is a significant conformational change to allow the NH\(_2\)-terminal lobe of the ELC to interact with the

**FIG. 4.** Enzymatic behavior of myosin containing mutant ELCs as functions of actin concentration. Actin-activated ATPase activity was assayed in varying concentrations of actin (0.125–8 \(\mu\)M). Myosin was treated with myosin light chain kinase prior to assays.

**FIG. 5.** Sliding velocities of actin filaments generated by wild type (wt) and mutant myosins. The kinase-treated myosin was measured to determine the velocity of actin filament motility in an in vitro motility assay. Greater than 70% of the filaments were observed to move in the fields recorded for measurement. Values are means and standard deviations of at least 10 filaments.

**FIG. 6.** Nuclear morphology of cells expressing ELC charge mutants. Cells were grown in suspension for 3 days and stained with DAPI (panels b, d, f, and h) to reveal the number of nuclei/cell. Left panels (a, c, e, and g) are phase-contrast images. Panels a and b, wild type cells; panels c and d, L1 ELC mutant; panels e and f, L2 ELC mutant; panels g and h, L3 ELC mutant.
motor domain of the HC, or there is a cooperativity between the two lobes of the ELC during the ATP hydrolysis. It is difficult to distinguish between these two possibilities with biophysical methods currently available. However, a gross S1 shape transition at its distal region, where the ELC resides, has been suggested to occur during the ATP hydrolysis based on modeling of small angle synchrotron x-ray scattering data (48). A major domain-domain rearrangement such as would occur in this scenario has been observed in the crystal structures of elongation factor Tu during the binding and release of nucleotide (53). Why the polar loops of the ELC have the observed effect on the actin-ATPase is not clear. One possibility is that because of their flexible nature, the polar loops could represent surfaces for domain-domain interactions without altering the folding of the main chain of the individual domains, which otherwise would not be possible. Such interdomain interactions at polar interfaces are evident in the domain rearrangements of elongation factor Tu (53).

Transient kinetic studies of the ATP hydrolysis of myosin establishes that ATP binding and hydrolysis occur at fast rates to produce ADP and P1 at the active site and that slow product release limits the steady-state rate in the absence of actin (54, 55). Although myosin carrying the ELC charge mutations does not affect the affinity for actin, this does not preclude the possibility that the ELC acts to facilitate the product release at the active site induced by actin binding. Detailed kinetic studies are necessary to dissect the role of ELC in the process.

The data presented here suggest that the ELC in a non-muscle system may play a significant role in determining the rate of actin-activated ATP hydrolysis. These are in contrast to its less significant role observed in skeletal muscle myosin (20, 21). It is interesting that the RLC regulates the actin-activated ATPase of both smooth muscle and non-muscle myosins but not the skeletal myosin (19), a feature that seems to correlate with functional significance of their counterpart ELC in these systems. Regulation of skeletal muscle myosin is governed by the thin filament-associated troponin-tropomyosin system. It is possible that in smooth muscle and non-muscle systems where myosin is regulated by its RLC subunit, the ELC may participate in more aspects of myosin function than merely as a structural support, especially considering the fact that it is located between the RLC and the active site (7, 8).