Identification and functional analysis of the essential and regulatory light chains of the only type II myosin Myo1p in Saccharomyces cerevisiae

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Cytokinesis in Saccharomyces cerevisiae involves coordination between actomyosin ring contraction and septum formation and/or targeted membrane deposition. We show that Mlc1p, a light chain for Myo2p (type V myosin) and Iqg1p (IQGAP), is the essential light chain for Myo1p, the only type II myosin in S. cerevisiae. However, disruption or reduction of Mlc1p–Myo1p interaction by deleting the Mlc1p binding site on Myo1p or by a point mutation in MLC1, mlc1-93, did not cause any obvious defect in cytokinesis. In contrast, a different point mutation, mlc1-11, displayed defects in cytokinesis and in interactions with Myo2p and Iqg1p. These data suggest that the major function of the Mlc1p–Myo1p interaction is not to regulate Myo1p activity but that Mlc1p may interact with Myo1p, Iqg1p, and Myo2p to coordinate actin ring formation and targeted membrane deposition during cytokinesis. We also identify Mlc2p as the regulatory light chain for Myo1p and demonstrate its role in Myo1p ring disassembly, a function likely conserved among eukaryotes.

Introduction

Cytokinesis is the last event in the cell division cycle and likely occurs by the contraction of a cortical actomyosin ring, which consists of type II myosin and F-actin. This contraction mechanism must be coordinated with membrane deposition at the cleavage site to ensure efficient cytokinesis and cell separation. Analysis of cytokinesis in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe has demonstrated that the basic mechanisms underlying cytokinesis are conserved between yeast and animal cells.

Each heavy chain of the type II myosin binds to two light chains, an essential light chain (ELC) proximal to the myosin head and a regulatory light chain (RLC) downstream of the ELC. These interactions are mediated by two tandem IQ motifs in the heavy chain. The ELC in Dictyostelium discoideum is required for cytokinesis and may regulate the actin-activated ATPase activity and stability of myosin filaments (Sellers, 1991; Trybus, 1991; Matsumura et al., 1998). Studies in D. discoideum and S. pombe suggest the binding of RLC to the heavy chain relieves an auto-inhibitory function of the RLC binding site as deletion of the relevant IQ site on the heavy chain suppresses defects associated with mutations in the RLC (Uyeda and Spudich, 1993; Naqvi et al., 2000). Myosin II molecules purified from RLC null cells in D. discoideum assemble into thick filaments with normal kinetics, but display a defect in filament disassembly in vitro (Chen et al., 1994). This defect has not been confirmed by in vivo studies.

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Abbreviations used in this paper: coIP, coimmunoprecipitation; DIC, differential interference contrast; ELC, essential light chain; RLC, regulatory light chain; SC, synthetic complete.

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Unlike the case in most animal cells and S. pombe, deletion of MYO1, which encodes the only type II myosin in S. cerevisiae, causes a defect in cytokinesis and cell separation, but not lethality, suggesting that actomyosin ring-independent mechanisms can carry out cytokinesis, albeit less efficiently (Bi et al., 1998; Vallen et al., 2000; Tolliday et al., 2003). Further studies suggest that this alternative mechanism may involve septum formation and/or targeted secretion. In contrast to MYO1, deletion of IQG1/CYK1, which encodes the only IQGAP in S. cerevisiae, or deletion of MLC1, which encodes a light chain for the type V myosin Myo2p and for Iqg1p, causes lethality with cells arrested in cytokinesis (Epp and Chant, 1997; Stevens and Davis, 1998; Lippincott and Li, 1998a; Shannon and Li, 2000). Mlc1p is required for the recruitment of Iqg1p to the bud neck (Shannon and Li, 2000), which, in turn, is required for actin ring formation (Epp and Chant, 1997; Lippincott and Li, 1998a). Because Iqg1p and Mlc1p are essential, they must play a role in the alternative mechanism in cytokinesis in addition to a role in actomyosin ring function.

Myo1p in S. cerevisiae contains two noncanonical IQ motifs. The light chains for Myo1p have not previously been identified, although Mlc1p is communoprecipitated with Myo1p (Boyne et al., 2000). We demonstrate here that Mlc1p is the ELC for Myo1p. However, binding of Mlc1p to Myo1p does not appear to play a major role in regulating Myo1p, but instead Mlc1p interacts with Myo1p, Iqg1p, and Myo2p to regulate actin ring formation and targeted secretion. In addition, we identify and demonstrate that Mlc2p is the RLC for Myo1p and that Mlc2p most likely plays a role in the disassembly of the Myo1p ring in vivo.

Results
Identification of Mlc2p
To identify the light chains for Myo1p, the head domain of Myo1p containing two putative IQ motifs (Fig. 1 A) was used in a two-hybrid screen against the yeast ORF-Gal4p activation domain fusion array to identify interacting proteins. Only one protein, encoded by YPR188C, carried the bait plasmid pOBD (DBD vector) or pOBD-MYO1-N (DBD-MYO1-N) was crossed with strain PJ69-4a carrying the prey plasmid pOAD alone or carrying one of its derivatives that contain one of the six calmodulin-related genes in S. cerevisiae: MLC2, MLC1, CMD1, FRQ1, CDC31, and CNB1. Diploids from the mating reactions were selected on SC-Trp-Leu and then replica-plated onto SC-His for detecting positive interactions. (A) Alignment of Mlc2p with other myosin II RLCs. The putative phosphorylation site, Ser6, is indicated by an asterisk and the EF hand is marked with a top line. Scer, from S. cerevisiae; Scas, from S. castellii; Sp, from S. pombe; and Hs, from Homo sapiens. Sequences are aligned with MacVector software.
plasmids were transformed into a
neck localization of Myo1p was not affected by deletion of
Myo1p-IQ1 localized normally in cells containing Myo1p-R806A or
studies of Mlc2p in various IQ mutants of Myo1p. Mlc2p
interact with Myo1p deleted for both IQ1 and IQ2 (Fig. 3 B).
As expected, Mlc2p did not in-
tween Mlc2p and Myo1p was abolished completely when
sins to alanine (Fig. 1 A). In contrast, the interaction be-
the highly conserved arginine among all known type II myo-
point mutation at residue 806 [R806A (IQ1)] that changes
Myo1p, Myo1p without IQ1 (IQ1
munoprecipitated effectively with GFP-tagged full-length
periments. As shown in Fig. 3 B, MYC-tagged Mlc2p coimmu-
ator control and were tagged
with GFP immediately after their start codon. Centromere-
based plasmids carrying these myo1 alleles along with control
plasmids were transformed into a myo1Δ MLC2:MYC strain, individually, for coimmunoprecipitation (coIP) ex-
periments. As shown in Fig. 3 B, MYC-tagged Mlc2p coim-
munoprecipitated effectively with GFP-tagged full-length
Myo1p, Myo1p without IQ1 (IQ1Δ), and Myo1p with a
point mutation at residue 806 [R806A (IQ1)] that changes
the highly conserved arginine among all known type II myo-
sins to alanine (Fig. 1 A). In contrast, the interaction be-
 tween Mlc2p and Myo1p was abolished completely when
IQ2 was deleted (Fig. 3 B). As expected, Mlc2p did not in-
teract with Myo1p deleted for both IQ1 and IQ2 (Fig. 3 B).
The coIP results correlate perfectly with the localization
studies of Mlc2p in various IQ mutants of Myo1p. Mlc2p
localized normally in cells containing Myo1p-R806A or
Myo1p-IQ1Δ as the sole source of Myo1p (Fig. 3 C). In con-
trast, the neck localization of Mlc2p was abolished completely
in cells containing Myo1p-IQ2Δ or Myo1p-(IQ1Δ+IQ2Δ) (Fig. 3 C), even though these mutant forms of MYO1 were
expressed at a similar level as the corresponding wild type, and
the Myo1p mutants themselves localized normally to the bud
neck (Fig. 3 B and not depicted). This result is similar to those
seen in S. pombe where deletion of the RLC binding site on
type II myosins abolishes the localization of RLC to the divi-
sion site (Naqvi et al., 2000). Together, the coIP results and
the localization studies indicate that Mlc2p binds to Myo1p
exclusively through IQ2. Based on the fact that RLCs of all
known type II myosins bind to their heavy chains through
IQ2, Mlc2p is likely a bona fide RLC for Myo1p.

Mlc2p localizes to the bud neck in a
Myo1p-dependent manner
If Mlc2p were a light chain for Myo1p, Mlc2p should show
similarity in its localization to Myo1p. Indeed, Mlc2p is the
only known protein that displays an identical localization
profile in the cell cycle as Myo1p. Mlc2p first localized to
the presumptive bud site as a cortical ring (Fig. 2 A, cell 1).
Once the bud emerged, Mlc2p formed a ring at the bud
neck (Fig. 2 A, cells 2 and 3). The Mlc2p ring maintained its
diameter at the neck until late anaphase when the Mlc2p
ring started to contract. The contraction process, which took
8–10 min (Fig. 2 B, bottom), was followed closely by the
centrifugal septum formation (Fig. 2 B, top), a behavior very
similar to that of Myo1p (Bi et al., 1998; Lippincott and Li,
1998a). The neck localization of Mlc2p was completely
abolished in myo1Δ cells (Fig. 2 C, middle). In contrast, the
neck localization of Myo1p was not affected by deletion of
MLC2 (analyzed in detail later; Fig. 2 C, right). These re-

Mlc2p binds to IQ2 of Myo1p and thus defines
an RLC for Myo1p
To determine the binding site of Mlc2p on Myo1p, we gen-
erated Myo1p mutants with a precise deletion of IQ1
(amino acids 795–815), IQ2 (amino acids 827–850), or
both (amino acids 795–850; Fig. 3 A). All these variants
were analyzed by time-lapse microscopy at 20°C. (A) Locali-
sation of Mlc2p to the bud neck and its dependency
on Myo1p. (A) Localization of Mlc2p in the cell cycle. Cells of
YEF2474 (MLC2-GFP/MLC2-GFP) grown exponentially in YM-P were
fixed with formaldehyde and visualized by DIC and fluores-
cence microscopy. (B) Contraction of Mlc2p-GFP ring during late anaphase
of the cell cycle. Cells of YEF2474 grown exponentially in SC medium
were analyzed by time-lapse microscopy at 20°C. (C) Localization
of Mlc2p to the bud neck depends on Myo1p. Localization of Mlc2p in
YEF2565 (myo1Δ/myo1Δ MLC2-GFP/MLC2-GFP) with (left) or
without (middle) the plasmid YCP50-MYO1 and localization of
Myo1p in YEF2616 (mlc2Δ/mlc2Δ MYO1-GFP/MYO1-GFP; right) were examined after streaking the strains onto YPD plates and
incubating the plates at 24°C for 12–16 h.
Mlc2p plays a role in the disassembly of the Myo1p ring in vivo

Surprisingly, deletion of MLC2 did not produce any obvious defects in growth rate, cytokinesis, and cell separation at temperatures ranging from 18 to 37°C on plates containing synthetic complete (SC) media or YPD media in the presence or absence of 1 M sorbitol or 0.9 M KCl, except that occasionally mlc2Δ cells formed cell clusters containing more than three cell bodies (unpublished data). Consistent with the hypothesis that Mlc2p functions through Myo1p, deletion of MLC2 and MYO1 together did not produce any additive effect on cytokinesis and cell separation. In addition, deletion of MLC2 together with each of the known cytokinesis genes including BNI1, BNR1, HOF1, and CYK3 did not significantly enhance the phenotypes of the single mutants (unpublished data). Furthermore, screens against the ordered array of yeast deletion mutants at 30 and 37°C failed to identify any mutants that displayed synthetic–lethal or synthetic–sick interactions with mlc2Δ cells. These data suggest that there might be multiple genes sharing a role with Mlc2p in cytokinesis; or more likely, that Mlc2p plays a subtle role in cytokinesis under laboratory conditions.

Upon detailed analyses, we found that the rate of Myo1p ring contraction in mlc2Δ diploid cells was approximately the same as that of an isogenic wild-type strain, but that there was a defect in ring disassembly. In wild-type cells at 20°C, the Myo1p “dot” disappeared within 1 min at the end of contraction (n = 7). However, ~46% of the mlc2Δ diploid cells (n = 13) showed a 2 to 8 min delay in the disassembly of the Myo1p dot at the end of its contraction at 20°C. We also performed time-lapse analyses on the same strains at 37°C. Unfortunately, the Myo1p-GFP signal at 37°C was not strong enough to produce interpretable time-lapse series. To pursue the mutant phenotype further, we reasoned that if the Myo1p ring were enlarged, the effect of
Mlc1p is an ELC for Myo1p but deletion of its binding site on Myo1p does not appear to affect Myo1p function

Mlc1p is known to coimmunoprecipitate with Myo1p (Boyne et al., 2000), suggesting that it might be a light chain for Myo1p. To examine this possibility, we mapped the binding site of Mlc1p on Myo1p by colP experiments with various IQ mutants of Myo1p. In contrast to Mlc2p, Mlc1p binding to Myo1p-IQ1Δ, Myo1p-IQ2Δ, or Myo1p-IQ1Δ+IQ2Δ (Fig. 5 A) was not detectable. These data indicate that Mlc1p binds to Myo1p through IQ1 and thus likely defines an “ELC” for Myo1p.

Interestingly, deletion of IQ1 alone or together with IQ2 did not affect the rate of the Myo1p ring contraction (n = 8) or the disassembly of the Myo1p dot at the end of Myo1p contraction (only one out of eight cells showed a slight delay in the disassembly of the Myo1p dot, similar to the behavior of Myo1p-IQ2Δ cells described previously; Fig. 5 B). These data suggest that binding of Mlc1p to Myo1p does not appear to play a major role in regulating Myo1p function and that the neck region of Myo1p including both IQ motifs is not essential for Myo1p function. A similar relationship between the type II myosins and their ELC Cdc4p has been observed in the fission yeast S. pombe (D’souza et al., 2001).

Mlc1p targets to the bud neck in the absence of its interactions with Myo1p and Myo2p

Because Mlc1p localizes to the bud neck and plays an essential role in cytokinesis (Stevens and Davis, 1998; Shannon and Li, 2000; Wagner et al., 2002), understanding the mechanisms underlying its neck localization might facilitate understanding its function in cytokinesis in general. Previous studies showed that the neck localization of Mlc1p occurs in strains deleted for Myo1p and in strains deleted for the IQ motifs of Myo2p (Boyne et al., 2000; Shannon and Li, 2000; Wagner et al., 2002). In addition, although Mlc1p binds to Ig1p, Mlc1p localization to the bud neck occurs before and independently of Ig1p (Boyne et al., 2000). In contrast, bud tip localization of Mlc1p has been demon-
strated to largely depend on its interaction with the IQ motifs of Myo2p (Shannon and Li, 2000). Some of these results are confirmed here (Fig. 6). We hypothesized that the IQ motifs in Myo1p and Myo2 might function redundantly in recruiting Mlc1p to the bud neck. Therefore, we examined whether or not simultaneous elimination of Mlc1p interactions with Myo1p and Myo2p would abolish the localization of Mlc1p to the bud neck. If so, this might cause cells to arrest at cytokinesis and/or cell separation, which would explain the essential role of Mlc1p in cytokinesis. However, the myo1Δ myo2IQ6Δ double mutant was viable and Mlc1p localized to the bud neck in the double mutant, albeit less efficiently than in either single mutant (Fig. 6 A). These results suggest that some Mlc1p molecules can localize to the bud neck independently of their interaction with either Myo1p or Myo2p. Surprisingly, the percentage of cells showing bud tip localization of Mlc1p in the myo1Δ myo2IQ6Δ double mutant was increased in comparison to the myo2IQ6Δ single mutant (Fig. 6 A), suggesting that Myo1p might normally trap some Mlc1p molecules at the bud neck and prevent them from reaching the bud tip. The tip, but not the neck, localization of Mlc1p was completely eliminated in the myo1Δ myo2IQ6Δ myo4Δ triple mutant (Fig. 6 A). Because budding was normal in the triple mutant, which presumably reflects normal targeting of secretory vesicles to the daughter cell, these results suggest that Mlc1p is normally targeted to the bud tip solely through its association with the two type V myosins, Myo2p and Myo4p, which have been shown to play a similar role in the bud tip localization of calmodulin (Stevens and Davis, 1998). These results also suggest that Mlc1p is not directly associated with secretory vesicles in contrast to a recent suggestion (Wagner et al., 2002).

What targets Mlc1p to the bud neck in the absence of its interactions with Myo1p, Myo2p, and Myo4p? Previous studies suggest that intact secretory pathway and septsins may be involved in the bud neck localization of Mlc1p (Boyne et al., 2000; Shannon and Li, 2000; Wagner et al., 2002). However, a previous work does not distinguish tip localization of Mlc1p from its neck localization in scoring the percentage of cells with a polarized Mlc1p signal (Wagner et al., 2002). The role of septsins in targeting Mlc1p to the bud neck remains controversial. One paper (Shannon and Li, 2000) indicates that the neck localization of Mlc1p depends on septsins before anaphase, but is independent of the septsins in cells with separated nuclei (late anaphase or telophase). Another paper indicates that the neck localization of Mlc1p depends on septsins for the entire population of cells (Boyne et al., 2000). For these reasons, we reexamined the role of secretory pathway and the septsins in the neck localization of Mlc1p. Our work showed that the neck localization of Mlc1p was largely unaffected by sec18-1, which blocks vesicle fusion at all stages of the secretory pathway or by sec2-41 and sec4-8, which block secretion from the Golgi to plasma membrane (Fig. 6 B). However, the bud tip localization of Mlc1p in these mutants was severely affected (Fig. 6 B), suggesting that the sec mutations demonstrated the expected defect. Further, these data suggest that the neck localization of Mlc1p is independent of the secretory pathway, which is consistent with the conclusion reached with the myo1Δ myo2IQ6Δ myo4Δ triple mutant. Our work also showed that the neck localization of Mlc1p strongly depended on the septsins, as only 2% of the budded cells with a single nucleus and 11.1% of cells with separated nuclei had Mlc1p at the bud neck after shifting the temperature-sensitive septin mutant, cdc12-6, to 37°C for 30 min (Fig. 6 B). This result...
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is in good agreement with one of the previous studies (Boyne et al., 2000).

In summary, there are at least three ways by which Mlc1p is targeted to the bud neck: by interacting with Myo1p or Myo2p directly and by interacting with the septins through an undefined mechanism.

Specific alleles of MLC1 reveal the functions of different Mlc1p interactions

In a screen for mutations that displayed synthetic-lethal interactions with the deletion of HOF1, a gene involved in coupling actomyosin-ring function to septum formation (details of the screen will be described elsewhere; Kamei et al., 1998; Lippincott and Li, 1998b; Vallen et al., 2000), we identified two mutant alleles of MLC1, which contain a single point mutation that causes either a glycine-to-aspartic acid change at residue 114 (G114D; mlc1-11) or a glycine-to-glutamic acid change at residue 135 (G135E; mlc1-93). Both changes occurred on amino acids that are highly conserved among the calmodulin (or light chain) superfamily (Fig. 7 A). The mlc1-11 cells were temperature-sensitive for growth (unpublished data) and showed a severe defect in cytokinesis even at 24°C, as the undivided mlc1-11 cells still shared a cytoplasm indicated by the decoration of the plasma membrane by GFP-tagged Ras2p (Fig. 7, B and C). In contrast, the mlc1-93 cells did not show any obvious defect in cytokinesis at temperatures ranging from 20 to 37°C (Fig. 7 B and not depicted). To determine the molecular basis for the behavior of the mlc1 mutant alleles, we did the following experiments. First, we determined the localization of the mlc1 mutant proteins and their interaction with Myo2p. We found that the protein encoded by mlc1-11 failed to localize efficiently to the presumptive bud site and to the bud tip (Fig. 7 D), which is very similar to the localization of wild-type Mlc1p in a myo2-IQ6Δ strain (Shannon and Li, 2000; Fig. 6 and Fig. 7 D). These data suggest that mlc1-11p may be defective in interaction with Myo2p, which was confirmed by colP experiments (Fig. 8 A). We also found that mlc1-11p localized to the bud tip less efficiently than wild-type Mlc1p in myo2-IQ6Δ cells (Fig. 7 D, asterisks), suggesting that there must be another mechanism for targeting Mlc1p to the bud tip independently of the IQ motifs of Myo2p, in which mlc1-11p is deficient. This conclusion is supported by the fact that the bud tip localization of Mlc1p largely depends on septin func-
played a similar reduced interaction with Myo1p (Fig. 8 C).

Next, we determined the effect of the mlc1 mutations on cytokinesis (Fig. 7 D). In contrast to mlc1-11p, mlc1-93p localized normally to the bud neck, indicating a defect in the Iqg1p–Mlc1p interaction, which was again confirmed by coIP experiments (Fig. 8 D). This fact that both mutant proteins localized normally to the bud neck suggests that the mechanism underlying the septin-dependent neck localization of Mlc1p is still largely intact and that mlc1-11p is unable to recruit or maintain Iqg1p at the neck. Consistent with this separation of functions for Mlc1p is the observation that a mutation at the corresponding position as mlc1-11 in Cdc4p, the ELC for type II myosins in S. pombe, also causes a temperature-sensitive cytokinesis defect without affecting the overall conformation of the protein (Slupsky et al., 2001). Because mlc1-93 cells did not show any obvious defect in cytokinesis in an otherwise wild-type background, these results lend further support to our previous notion that the Myo1p–Mlc1p interaction may not play a major role in regulating Myo1p function or cytokinesis. Because elimination of the Mlc1p–Myo2p interaction by deletion of the IQ motifs of Myo2p (Stevens and Davis, 1998) also does not cause any obvious defect in cytokinesis, the genotype of mlc1-11 cells suggests that the cytokinesis defect is mainly due to its defective interaction with Iqg1p or due to a combinatorial effect of its decreased interactions with all the binding partners. Strikingly, the mlc1-11 mutation (Fig. 8 E, green) mapped on the three-dimensional structure of IQ2 (from Myo2p)–Mlc1p complex (Terrak et al., 2003) at the interface between the IQ motif and the COOH-terminal domain of Mlc1p, whereas the mlc1-93 mutation (Fig. 8 E, red) was mapped at a site quite distal to the IQ2–Mlc1p interface. Previous data has demonstrated that alleles of CDC4 in S. pombe display intragenic complementation (Nurse and Nasmyth, 1976). Our data, taken with the structural studies (Slupsky et al., 2001; Terrak et al., 2003) suggest that Mlc1p interactions with the IQ regions of Myo1p and Myo2p and/or Iqg1p may be mediated, at least in part, through distinct domains and thereby explains the intragenic complementation of some ELC alleles.

**Discussion**

Mlc2p regulates the disassembly of the Myo1p ring

We have shown that Mlc2p shares a localization and contraction profile in the cell cycle with Myo1p and that the neck localization of Mlc2p completely depends on Myo1p. In addition, coIP and localization experiments indicate that Mlc2p binds to Myo1p exclusively through the IQ2 motif of Myo1p; thus defining Mlc2p as the RLC for Myo1p.

Deletion of MLC2 produced a mild but consistent defect in the disassembly of the Myo1p ring, at least at the end of its contraction. Because the Myo1p ring is small, even in tetraploid cells, we cannot rule out the possibility that there also might be a subtle change in the contraction kinetics in mlc2Δ cells that we could not detect. In fact, a defect in contraction could certainly arise from a defect in disassembly because these processes are normally coupled (Schroeder, 1972). The biochemical basis for the Myo1p disassembly defect is not clear. However, the type II myosin purified from D. discoideum RLC null cells formed thick filaments comparable to wild-type myosin, but displayed a defect in

In summary, mlc1-11p is defective in its interaction with Myo2p and Iqg1p; and both mlc1-11p and mlc1-93p display a reduced interaction with Myo1p (Fig. 8 D). The fact that both mutant proteins localized normally to the bud neck suggests that the mechanism underlying the septin-dependent neck localization of Mlc1p is still largely intact and that mlc1-11p is unable to recruit or maintain Iqg1p at the neck.
the disassembly properties in vitro (Chen et al., 1994). These data suggest that the basic function of an RLC may be conserved through evolution. Further biochemical studies on different pairs of RLC–heavy chain interactions are required to determine whether or not a common molecular mechanism underlies the similar function for the RLCs.

The family of RLCs for nonmuscle type II myosins appears to have three features. First, they share very limited sequence homology. For example, Mlc2p displays only 19% identity and 38% similarity in amino acid sequence with Rlc1p, the RLC in S. pombe. Second, the requirement for the function of the RLC in modulating the heavy-chain activity and thus in cytokinesis appears to increase with organismal complexity. In Drosophila melanogaster, mutations in the RLC gene spaghetti-squash cause embryonic lethality and a severe defect in cytokinesis (Karess et al., 1991). In D. discoideum, deletion of the RLC gene does not cause cell lethality, but produces a cytokinesis defect similar to that caused by deletion of the heavy chain (Chen et al., 1994). In S. pombe, rlc1 is only needed for cell viability and cytokinesis at low temperature (Le Goff et al., 2000; Naqvi et al., 2000). In S. cerevisiae, deletion of MLC2 produces a mild defect in the disassembly of the Myo1p ring that is only detectable with a sensitive approach such as time-lapse microscopy. Finally, RLCs appear to act on their respective type II myosins only, which may explain why these light chains are so evolutionarily divergent.

Mlc1p may interact with Myo1p, Iqg1p, and Myo2p to coordinate the formation and contraction of the actomyosin ring with targeted membrane deposition

We show here that Mlc1p binds to Myo1p through the IQ1 motif of Myo1p, suggesting that Mlc1p is an ELC for Myo1p. Mlc1p is also a light chain for Myo2p (the type V myosin) and Iqg1p (IQGAP; Stevens and Davis, 1998; Shannon and Li, 2000). It is not clear how Mlc1p interacts with different proteins in a temporally and spatially regulated manner to carry out its essential function in cytokinesis. The relative contribution of each Mlc1p interaction to its role in cytokinesis can be assessed by analyzing the consequences of disrupting each specific interaction by mutations in Mlc1p or its binding partners.

First, the Mlc1p–Myo1p interaction does not appear to play a significant role in regulating Myo1p function, because deletion of IQ1 alone or together with IQ2 in Myo1p, which abolished the binding of Mlc1p to Myo1p, did not cause an obvious defect in cytokinesis. The phenotype of myo1-(IQ1Δ+IQ2Δ) is much less severe than complete deletion of MYO1 in this strain background, suggesting that Myo1p can perform some functions in cytokinesis in the absence of Mlc1p binding. This conclusion is further supported by the fact that a specific mutation in MLC1 (mlc1-93) that decreased the Mlc1p–Myo1p interaction, but maintained the Mlc1p–Iqg1p and the Mlc1p–Myo2p interactions, did not show any obvious defect in cytokinesis either. Second, the Mlc1p–Myo2p interaction is not responsible for the major role of Mlc1p in cytokinesis because a myo2-1IQ6Δ strain, in which Mlc1p does not interact with Myo2p directly (Stevens and Davis, 1998), is able to carry out cytokinesis to a large degree, although it may be slightly defective in cytokinesis and/or cell separation. Even simultaneous disruption of the Mlc1p–Myo1p and the Mlc1p–Myo2p interactions in a myo1-(IQ1Δ+IQ2Δ) myo2-1IQ6Δ strain did not cause a defect in cytokinesis nearly as severe as the depletion of Mlc1p, although the double mutant showed a slight additive defect in cytokinesis (unpublished data). These data suggest that the interaction of Mlc1p with Myo1p and Myo2p plays a somewhat minor role in cytokinesis.

In contrast, the Mlc1p–Iqg1p interaction appears to play a major role in cytokinesis. A specific mutation in MLC1 (mlc1-11), which causes a defect in its interactions with Myo1p, Myo2p, and Iqg1p, is defective in cytokinesis. Because the Mlc1p–Myo1p and Mlc1p–Myo2p interactions play only a fine-tuning role in cytokinesis, the major role of Mlc1p in cytokinesis is likely performed through its interaction with Iqg1p. Mlc1p is required for the recruitment of Iqg1p to the bud neck (Boyne et al., 2000; Shannon and Li, 2000), which, in turn, is required for actin ring formation (Epp and Chant, 1997; Lippincott and Li, 1998a); thus, Mlc1p–Iqg1p interaction plays an essential role in the assembly of the actomyosin ring. Both Mlc1p and Iqg1p must play an additional role in cytokinesis independent of their role in actomyosin ring assembly because deletion of MLC1 or IQG1 causes cell lethality with cells accumulating in chains, whereas deletion of MYO1, which abolishes the actomyosin ring function (Bi et al., 1998), in the same strain background does not (Boyne et al., 2000; unpublished data). Indeed, multicopy HOF1 or CYK3 can suppress an iqg1 deletion and its associated cytokinesis defect without restoring the actomyosin ring function (Korinek et al., 2000). The actomyosin ring-independent function of Iqg1p in cytokinesis could involve targeted secretion to the bud neck and/or septum formation (Korinek et al., 2000; Bi, 2001; Osman et al., 2002).

In contrast to RLCs, the sequences and functions of the ELCs of the type II myosins appear to be better conserved through evolution. For example, Mlc1p shares 42% identity and 61% similarity in amino acid sequence with Cdc4p, the ELC of type II myosins in S. pombe. In addition, all known ELCs including Cdc4p in S. pombe, Mlc1p in S. cerevisiae, and ELC in D. discoideum play an essential role or as important a role as their respective heavy chains in cytokinesis (Pollenz et al., 1992; McCollum et al., 1995; Stevens and Davis, 1998; D’souza et al., 2001). Like Mlc1p, Cdc4p in S. pombe also appears to interact with the IQGAP and a type V myosin to execute its function in cytokinesis (D’souza et al., 2001; Win et al., 2001). The interactions of the ELCs with other conserved molecules may act as an evolutionary constraint and explain the higher conservation among the ELCs from different organisms.

An integrated view of cytokinesis in S. cerevisiae

Increasing evidence suggests that cytokinesis in animal cells and in fungi involves interplay between actomyosin ring function and targeted secretion to the division site (Hales et al., 1999; Shuster and Burgess, 2002; Wang et al., 2002). In S. cerevisiae, cytokinesis involves the coordinated action of the actomyosin ring and septum formation (Fig. 9 A), which probably requires targeted secretion (Vallen et al., 2000; Bi,
The role of the ELC in promoting actomyosin ring formation through type II myosin and IQGAP and in coordinating the actomyosin ring contraction with targeted secretion through type V myosin, and perhaps IQGAP, appears to be a conserved feature in cytokinesis between *S. cerevisiae* and *S. pombe*, two distantly related fungi, although many details of biochemical interactions require further investigation. Because all the major molecules involved in these processes are conserved through evolution, it is tempting to speculate that similar mechanisms involving the ELC may exist in animal cells.

**Materials and methods**

**Strains, growth conditions, and genetic methods**

Yeast strains are listed in Table 1. Standard culture media and genetic methods were used (Guthrie and Fink, 1991). In some cases, 1 mg/ml 5-fluoroorotic acid (Angus Buffers & Biochemicals) was added to media to select for loss of *URA3*-containing plasmids.

**Plasmids**

Plasmid pOBD-MYO1-N was constructed by cloning the *MYO1*-head (amino acids 1–855) coding sequence into pOBD by a gap repair-mediated method (Drees et al., 2001). Plasmids pOAD carrying different light chain-related genes used in Fig. 1 B were supplied by S. Fields’ group (University of Washington, Seattle, WA). Plasmid YpC50-MYO1 (CEN URA3; supplied by S. Brown, University of Michigan, Ann Arbor, MI) carries wild-type *MYO1* under its own promoter control. Plasmid pRS316-NoNot is a derivative of pRS316 (CEN URA3; Sikorski and Hieter, 1989), in which the unique NotI site has been destroyed (Caviston et al., 2003). Plasmid pRS316-MYC-MYO1 carries wild-type *MYO1* (Caviston et al., 2003). Plasmid pRS316-N-MYO1-GFP carries *MYO1* with a GFP cassette inserted in-frame after the start codon of *MYO1* (Caviston et al., 2003). Different IQ mutations (R806A, IQ1A, IQ2A, and IQ1A+IQ2A) of *MYO1* were introduced into pRS316-N-MYO1-GFP using a PCR-based method with appropriate mutagenic primers. Plasmid pRS315-GFP-RAS2 contains an ∼2.5-kb HindIII-XbaI fragment carrying GFP-RAS2 from plasmid pH192 (Philips and Herskowitz, 1997) that was cloned into the corresponding sites in pRS315 (CEN LEU2; Sikorski and Hieter, 1989). Plasmids pUG34-MLC1 (CEN HIS3) and pUG35-IQG1 (CEN URA3); supplied by A. Ragni-Wilson, University of “Tor Vergata” Rome, Italy) carrying MET25p-yEGFP-MLC1 and MET25p-yEGFP-IQG1, respectively, were described previously (Wagner et al., 2002). Plasmid pUG34 (supplied by J.H. Hedegermann, Heinrich-Heine-Universität, Düsseldorf, Germany) carrying *mlc1-11* or *mlc-93* was constructed similarly as pUG34-MLC1. A PCR fragment flanking the *MLC1* locus was amplified from the mutant strain carrying *mlc1-11* or *mlc1-93*. PCR fragments were mixed with Xmal-EcoRI-digested pUG34 and transformed into YEF473A for gap-repair to generate the desired plasmids. All mutagenic fragments were confirmed by DNA sequencing at the Sequencing Facility of the University of Pennsylvania. All oligonucleotide primers were purchased from Integrated DNA technologies.

**Construction of yeast strains**

Yeast strains carrying *MYO1*:GFP-Kan (YEF2293 and YEF2294), *MLC2*:GFP-Kan (YEF2455), *MLC2*:MYC-HIS3 (YEF2661), and *mlc1-93*:HIS3 (YEF2598) were constructed using a PCR-based method (Longtine et al., 1998), except that the *gfp* allele in the template plasmid pFA6a-GFP (S65T, F64L)-KanMX6 carries two mutations (Caviston et al., 2003). Strains YJL175A (CEN LEU2) and YJL176A (CEN LEU2; supplied by S. Brown, University of Michigan, Ann Arbor, MI) carrying *MLC2*:GFP-Kan (YEF2661), *MLC2*:MYC-Kan (YEF2293), and *MYO1*:GFP-Kan (YEF2294) were constructed using a PCR-based method (Longtine et al., 1998) to strains SCS550 and YEF473A, respectively.

To construct the tetraploid strains, *can1Δ::MFAP1pr-HIS3-Mla1pr-LEU2* was amplified from strain Y3656 (supplied by C. Boone, University of Toronto, Toronto, Canada) and transformed into YEF2293 (a MYO1:GFP-Kan), selecting for *His*<sup>+</sup> strains YEF3316 and YEF3317 that are heterozygous for the dual reporter locus. YEF3316 and YEF3317 were streaked onto SC-His and SC-Leu.
Table 1. Yeast strains used in this study

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<th>Strain</th>
<th>Genotype</th>
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<tr>
<td>JGY288</td>
<td>a sec2-41 his3 leu2 trp1 ura3</td>
<td>Caviston et al., 2003</td>
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<td>M-17</td>
<td>acdc12-6 leu2 ura3</td>
<td>Gao et al., 2003</td>
</tr>
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<td>Pi69-4a</td>
<td>a trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ lys2::gal1-HIS3 GAL2-ADE2 met2::gal7-lacZ</td>
<td>James et al., 1996</td>
</tr>
<tr>
<td>Pi69-4a</td>
<td>a trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ lys2::gal1-HIS3 GAL2-ADE2 met2::gal7-lacZ</td>
<td>James et al., 1996</td>
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<td>RSY21</td>
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<td>SEY6210</td>
<td>a his3 leu2 lys2 suc2 trp1 ura3</td>
<td>Burd et al., 1997</td>
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<td>Burd et al., 1997</td>
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<td>a can1Δ::MFA1pr-HIS3-Mfa1pr-LEU2</td>
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<td>YEF473</td>
<td>a his3 his3 leu2/leu2 lys2/lys2 trp1/1 ura3/ura3</td>
<td>Bi and Pringle, 1996</td>
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<td>a his3 leu2 lys2 trp1 ura3</td>
<td>Bi and Pringle, 1996</td>
</tr>
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<td>a myo1Δ::Kan his3 leu2 lys2 trp1 ura3</td>
<td>Bi et al., 1998</td>
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<td>Bi et al., 1998</td>
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<td>YEF2293</td>
<td>a MYO1::GFP-Kan his3 leu2 lys2 trp1 ura3</td>
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<td>YEF2293 X YEF2294</td>
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<td>YEF2455</td>
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<td>Segregant of YEF2455</td>
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<td>As YEF473 except myo1Δ::HIS3/MLC2::HIS3 MLC2::GFP-Kan/MLC2::GFP-Kan (YCps50-MYO1)</td>
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<td>YEF3235</td>
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<td>YEF3303</td>
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<td>YJL126</td>
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<tr>
<td>YJL176A</td>
<td>a MYO1::HA-TRP1 MYO2::MYC-Kan his3 leu2 lys2 trp1 ura3</td>
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</table>

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⁹YEF1751 (myo1Δ::HIS3/MYO1) carrying YCps50-MYO1 (Vallen et al., 2000) was sporulated to generate YEF2056.

⁸YEF2056 was crossed to YEF2473. Segregants with appropriate genotypes and opposite mating types were crossed to form YEF2293 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.

⁷A PCR fragment carrying mlc2Δ::HIS3 was amplified from YEF2598 and transformed into YEF2923 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.

⁶A PCR fragment carrying myo1Δ::Kan was amplified from YEF1804 and transformed into a segregant of YEF2661 to generate YEF3175.

⁵A PCR fragment carrying mlc2Δ::TRP1 was amplified from YEF1804 and transformed into SCC1 to yield YJL126.

₄A PCR fragment carrying mlc2Δ::TRP1 was amplified from YEF1804 and transformed into YEF2923 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.

³A PCR fragment carrying mlc2Δ::TRP1 was amplified from YEF1804 and transformed into YEF2923 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.

²A PCR fragment carrying myo1Δ::Kan was amplified from YEF1804 and transformed into a segregant of YEF2661 to generate YEF3175.

¹A PCR fragment carrying mlc2Δ::TRP1 was amplified from YEF1804 and transformed into YEF2923 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.

⁰A PCR fragment carrying mlc2Δ::TRP1 was amplified from YEF1804 and transformed into YEF2923 and YEF2294, yielding YEF2612 and YEF2613, respectively, which were then crossed to generate YEF2616.
DeMarini, D.J., A.E.M. Adams, H. Fares, C. De Virgilio, G. Valle, J.S. Chuang, and analyzed using Image-Pro Plus software (Media Cybernetics). The con-

ual cells were followed at 1- or 2-min intervals using a computer-con-

Ura medium were spotted onto a microscope slide spread with a thin layer

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