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# Cytokinesis Depends on the Motor Domains of Myosin-II in Fission Yeast but Not in Budding Yeast

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**Budding yeast possesses one myosin-II, Myo1p, whereas fission yeast has two, Myo2p and Myp2p, all of which contribute to cytokinesis. We find that chimeras consisting of Myo2p or Myp2p motor domains fused to the tail of Myo1p are fully functional in supporting budding yeast cytokinesis. Remarkably, the tail alone of budding yeast Myo1p localizes to the contractile ring, supporting both its constriction and cytokinesis. In contrast, fission yeast Myo2p and Myp2p require both the catalytic head domain as well as tail domains for function, with the tails providing distinct functions (Bezanilla and Pollard, 2000). Myo1p is the first example of a myosin whose cellular function does not require a catalytic motor domain revealing a novel mechanism of action for budding yeast myosin-II independent of actin binding and ATPase activity.**

## INTRODUCTION

Cytokinesis, the physical separation of dividing cells, is a fundamental but poorly understood event. Formation of a cleavage furrow depends on the actin-based motor myosin-II to constrict a contractile ring of actin filaments (Mabuchi and Okuno, 1977). Genetic studies in budding and fission yeast (Guertin *et al*., 2002) and RNA interference experiments on animal cells (Somma *et al*., 2002; Kiger *et al*., 2003; Rogers *et al*., 2003; Echard *et al*., 2004) have identified a growing list of other genes required for cytokinesis.

The heavy chain of myosin-II forms an N-terminal catalytic domain (head) and a C-terminal coiled-coil tail. The head binds actin filaments and catalyzes ATP hydrolysis. The segment of the heavy chain between the catalytic domain and the tail binds two light chains related to calmodulin: an essential light chain (ELC) and a regulatory light chain (RLC). This light chain domain forms a lever arm that amplifies the force-producing conformational changes in the head. Phosphorylation of RLCs regulates the motor activity of animal cell myosin-II (Sellers *et al*., 1981; Bresnick, 1999), whereas an Unc45-/Cro1p-/She4p-related (UCS) protein regulates the motor activity of fission yeast myosin-II (Lord and Pollard, 2004). Myosin-II tails assemble into bipolar filaments, allowing contraction of actin filaments in muscle and other cells. The tails of nonmuscle myosin-II may have additional functions. The tails of myosin-II from *Dictyostelium* and *Acanthamoeba* have a role in recruitment to the cleavage furrow (Yumura and Uyeda, 1997; Zang and Spudich, 1998; Shu *et al*., 1999, 2003).

The fission yeast, *Schizosaccharomyces pombe*, has two myosin-II heavy chains, both paired with ELC Cdc4p (McCollum *et al*., 1995; Motegi *et al*., 2000; Naqvi *et al*., 2000; Lord *et al*., 2004) and RLC Rlc1p (Le Goff *et al*., 2000; Naqvi *et al*., 2000; Lord and Pollard, 2004). Myo2p is essential for cytokinesis and viability under most conditions (Kitayama *et al*., 1997; May *et al*., 1997; Balasubramanian *et al*., 1998), but Myp2p is required only under special stressful conditions (Bezanilla *et al*., 1997; Motegi *et al*., 1997). The tails specify unique functions, including localization of Myo2p and Myp2p at the contractile ring (Naqvi *et al*., 1999; Bezanilla and Pollard, 2000; Bezanilla *et al*., 2000). Myo2p is "conventional" in the sense that two heavy chains form a rod-shaped, coiled-coil tail that is insoluble at low ionic strength, presumably owing to the formation of filaments (Bezanilla and Pollard, 2000; Lord and Pollard, 2004). Myo2p is the second known contractile ring protein to concentrate around the equator of dividing cells, independent of actin filaments and septins, which arrive later (Naqvi *et al*., 1999; Wu *et al*., 2003). Accumulation of Myo2p at the cell equator is dependent on dephosphorylation of a phosphoserine at the C terminus of its tail (Motegi *et al*., 2004). Compaction of Myo2p into a tight contractile ring depends on actin filaments (Motegi *et al*., 2000; Wu *et al*., 2003) and Rng3p (Wong *et al*., 2000; Lord and Pollard, 2004). Myp2p is "unconventional," because the tail has a large insert between two segments of heptad repeats. This allows isolated tail polypeptides to fold back to form an antiparallel coiled-coil, indicating that Myp2p is monomeric with a single head (Bezanilla and Pollard, 2000). In spite of this unconventional structure, the tails are insoluble at physiological ionic strength. Myp2p joins the contractile ring 25 min after Myo2p (Wu *et al*., 2003) whereupon it acts to stabilize the contractile ring (Mulvihill and Hyams, 2003).

Budding yeast (*Saccharomyces cerevisiae*) myosin-II, Myo1p, contributes to cytokinesis and primary septum formation (Watts *et al*., 1987; Bi *et al*., 1998; Lippincott and Li, 1998; Schmidt *et al*., 2002). In certain strain backgrounds (e.g., BF264-Du), *myo1*Δ cells are viable despite morphological defects at the division site and failure of cytokinesis in some cells (Bi *et al*., 1998). In other backgrounds (e.g., W303a), the severity of cytokinesis defects results in lethality (Tolliday *et al*., 2003). Mlc1p is the ELC and Mlc2p is the RLC for Myo1p (Luo *et al*., 2004). Like the Myp2p tail, the Myo1p

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Abbreviations used: ELC, essential light chain; RLC, regulatory light chain; UCS, Unc-45-/Cro1p-/She4p-related.

**Table 1.** Yeast strains employed in this study

| Strain | Genotype | Source |
|---|---|---|
| Budding yeast | | |
| JC 19 | *MATα sst2* | J. Chant |
| JC 65 | *MAT***a** *bar1* | J. Chant |
| JC 1284 | *MATα leu2-3 his3-11 ura3-52 lys2 trp1-1 suc2-Δ9* | J. Chant |
| JC 1401 | *MATα leu2-3 his3-11 ura3-52 ade2-1 trp1-1 she4Δ::URA3* | J. Chant |
| JC 1609 | *MAT***a** *leu2-3 his3-11 ura3-52 ade2-1 trp1-1 suc2-Δ 9 myo1Δ::HIS3* (BF264-Du background) | J. Chant |
| EL 51 | *MATα leu2-3 his3-11 ura3-52 ade2-1 trp1-1 myo1Δ::HIS3 she4Δ::URA3* | Segregant of JC 1401 × JC 1609 |
| RLY 1236 | *MAT***a**/*α leu2-3/leu2-3 his3-11/his3-11 ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 bar1Δ/bar1Δ myo1Δ::kan$^R$/MYO1* (W303a background) | Tolliday *et al*. (2003) |
| Fission yeast | | |
| FY 527 | *h$^-$ leu1-32 his3-D1 ura4-D18 ade6-M216* | S. Forsburg |
| TP 5 | *h$^-$ leu1-32 his7-366 ura4-D18 ade6-M210 myp2Δ::his7$^+$* | Bezanilla *et al*. (1997) |
| TP 73 | *h$^-$ leu1-32 his7-366 ura4D18 ade6-M216 myo2-E1* | M. Balasubramanian |
| JW 994 | *h$^-$ leu1-32 his3-D1 ura4-D18 ade6-M216 kan$^R$:GFP-myp2* | Wu *et al*. (2003) |
| EL 101 | *h$^-$ leu1-32 his3-D1 ura4-D18 ade6-M216 kan$^R$:GFP-Δ$^{bp1-2547}$myp2* | This study |
| EL 102 | *h$^-$ leu1-32 his3-D1 ura4-D18 ade6-M216 kan$^R$:GFP-myp2Δ$^{bp2548-6312}$:nat$^R$* | This study |

tail is relatively long, with two distinct coiled-coil regions separated by a segment unlikely to form a coiled-coil (Bezanilla and Pollard, 2000). No information is available on the physical properties of the Myo1p tail. Concentration of Myo1p in the contractile ring early in the cell cycle at $G_1$/S phase depends on the septin protein complex but not on actin filaments (Bi *et al*., 1998; Lippincott and Li, 1998).

We investigated the functional relationships among Myo1p, Myo2p, and Myp2p by using complementation and localization experiments. Remarkably, the Myo1p tail alone fully supports localization to the contractile ring and cytokinesis of both the BF264-Du and W303a strains of budding yeast. In contrast, both the heads and tails of Myo2p and Myp2p are necessary for their function in fission yeast. Our work reports the first known case of a myosin that maintains its cellular function without a motor domain and highlights the importance of myosin-II tails in cytokinesis.

## MATERIALS AND METHODS

### *Yeast Strains, Growth Conditions, Cell, and Genetic Methods*

Table 1 lists budding and fission yeast strains. Standard budding and fission yeast growth conditions, genetic methods, and DNA staining procedures

**Table 2.** Plasmids employed in this study

| Plasmid | Comment | Source |
|---|---|---|
| *pFA6a-kanMX6* | | Bahler *et al*. (1998) |
| *pFA6a-myp2 promoter-GFP-kanMX6* | | Wu *et al*. (2003) |
| *pFA6a-natMX4* | | This study |
| *pAG36* | *natMX4* in pRS316 derivative | Goldstein and McCusker (1999) |
| Budding yeast plasmids | | |
| *YCp50-MYO1* | *MYO1 clone* | J. Chant |
| *pRS-314* | *TRP1*, low copy, centromeric vector | Sikorski and Hieter (1989) |
| *pPGT* | *MYO1* promoter-*GFP-MYO1* terminator in *pRS314* | This study |
| *pGFP-MYO1* | *pPGT* encoding modular full-length Myo1p | This study |
| *pGFP-myo2* | *pPGT* encoding full-length Myo2p | This study |
| *pGFP-myp2* | *pPGT* encoding full-length Myp2p | This study |
| *pGFP-MYO1H-myo2T* | *pPGT* encoding Myo1p head-Myo2p tail chimera | This study |
| *pGFP-MYO1H-myp2T* | *pPGT* encoding Myo1p head-Myp2p tail chimera | This study |
| *pGFP-myo2H-MYO1T* | *pPGT* encoding Myo2p head-Myo1p tail chimera | This study |
| *pGFP-myp2H-MYO1T* | *pPGT* encoding Myp2p head-Myo1p tail chimera | This study |
| *pGFP-MYO1T* | *pPGT* encoding Myo1p tail alone (aa 842–1928) | This study |
| Fission yeast plasmids | | |
| *p572-81X* | *ura4*, 81*nmt1* promoter-C-terminal *GFP* vector | S. Forsburg |
| *p573-81X* | *LEU2*, 81*nmt1* promoter-N-terminal *GFP* vector | S. Forsburg |
| *p81-myo2* | *p573-81X* encoding full-length Myo2p | Bezanilla and Pollard (2000) |
| *p81-myp2* | *p573-81X* encoding full-length Myp2p | Bezanilla and Pollard (2000) |
| *p81-myo2H* | *p572-81X* encoding Myo2p head alone (aa 1–815) | This study |
| *p81-myo2H-myp2T* | *p573-81X* encoding Myo2p head-Myp2p tail chimera | Bezanilla and Pollard (2000) |
| *p81-myp2H-myo2T* | *p573-81X* encoding Myp2p head-Myo2p tail chimera | Bezanilla and Pollard (2000) |
| *p81-MYO1* | *p573-81X* encoding full-length Myo1p | This study |
| *p81-myo2H-MYO1T* | *p573-81X* encoding Myo2p head-Myo1p tail chimera | This study |
| *p81-MYO1H-myo2T* | *p573-81X* encoding Myo1p head-Myo2p tail chimera | This study |
| *p81-myp2H-MYO1T* | *p573-81X* encoding Myp2p head-Myo1p tail chimera | This study |
| *p81-MYO1H-myp2T* | *p573-81X* encoding Myo1p head-Myp2p tail chimera | This study |

were used (Rose *et al*., 1990; Moreno *et al*., 1991). We used two different *myo1*Δ strains with different genetic backgrounds: JC 1609 in the BF264-Du background, in which deletion of *MYO1* is not lethal; and RLY 1236 in the W303a background, in which *MYO1* is essential (Tolliday *et al*., 2003). We used the JC1609 strain for complementation experiments using haploids. Derivatives of JC 1609 (referred to as JC 1609 for simplicity) were used after thorough backcrossing with a congenic wild-type strain to eliminate the emergence of suppressors, which are known to arise in this *myo1*Δ haploid background (Tolliday *et al*., 2003). To test the ability of constructs to rescue in a background where *MYO1* represents an essential gene, we transformed a heterozygote diploid (RLY 1236 *myo1*Δ:*kan*$^R$/*MYO1*) with appropriate plasmids. Transformants were sporulated and tetrads were dissected onto YPDa plates. Segregants were further tested for geneticin resistance (*kan*$^R$) on YPDa plates containing 200 mg/ml geneticin (Invitrogen, Carlsbad, CA), plasmid possession on CSM-Trp$^-$ plates, and mating type by halo assays using **a**- (JC 19) and α-factor (JC 65) tester strains. Budding yeast strains carrying green fluorescent protein (GFP) fusion protein plasmids were used in complementation and localization experiments after growing exponentially in CSM-Trp$^-$ medium.

Fission yeast strains carrying GFP fusion protein plasmids were selected on EMM minus leucine plates containing 5 μg/ml thiamine. Cells were used in experiments after growing exponentially in EMM minus leucine plus 5 μg/ml thiamine (repressing conditions for complementation experiments) or plus 5–50 ng/ml thiamine (semirepressing conditions for localization experiments).

Truncation and GFP tagging of chromosomal *myp2* was achieved using the one-step PCR and homologous integration method of Bahler *et al*. (1998). The geneticin-resistance gene (*kan*$^R$) and nourseothricin-resistance gene (*nat*$^R$) were used to truncate *myp2* at the N terminus ("headless") and C terminus ("tailless"), respectively. The N-terminal truncation resulted in replacement of the first 849 amino acids of Myp2p with GFP, whereas the C-terminal truncation resulted in removal of the last 1255 amino acids. Oligonucleotides containing *kan*$^R$ or *nat*$^R$ were integrated into chosen strains through high efficiency transformation. Integrants were subsequently selected on YE5S plates containing 100 μg/ml geneticin or 50 μg/ml clonNAT (Werner Bioreagents, Hamburg, Germany). Oligonucleotides were amplified with a High Fidelity polymerase (Roche Diagnostics, Indianapolis, IN) from *pFA6a-myp2 promoter-GFP-kanMX6* or *pFA6a-natMX4* accordingly. Correct integration was confirmed by diagnostic PCR of genomic DNA using oligonucleotides derived from the sequence of selective markers and the target site. The phenotype and localization of integrated Myp2p GFP fusion proteins were analyzed after exponential growth in EMM medium (20°C) and YE5S medium (30°C), respectively.

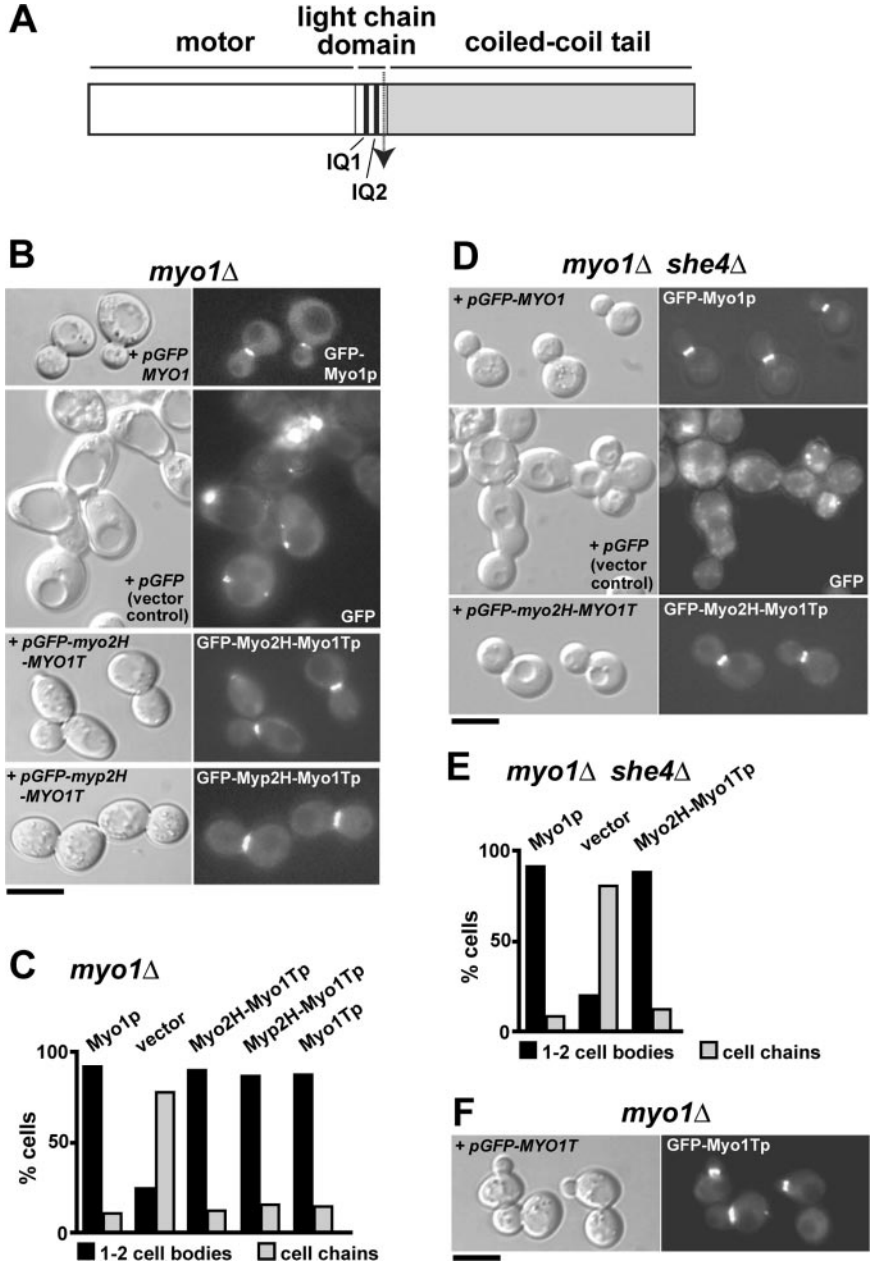


**Figure 1.** The Myo1p tail alone provides the full function of Myo1p during cytokinesis in the BF264-Du strain background. (A) Diagram of the myosin-II domain structure shown for a hypothetical myosin-II sequence. The N-terminal catalytic domain or head binds ATP and actin filaments. The light chain domain binds the ELC (IQ1) and RLC (IQ2). The C-terminal tail forms a dimer that assembles into bipolar filaments. The arrow indicates the site at the beginning of the tail where an *NcoI* site was engineered into the coding sequence of *MYO1*, *myo2*, and *myp2* to facilitate construction of plasmids containing chimeras and the headless Myo1Tp (lacking both the catalytic and light chain domains). (B) Differential interference contrast (DIC) (left) and fluorescence (right) micrographs of budding yeast *myo1*Δ cells expressing GFP-Myo1p (*pGFP-MYO1*), GFP (*pPGT*), GFP-Myo2H-Myo1Tp (*pGFP-myo2H-MYO1T*), or GFP-Myp2H-Myo1Tp (*pGFP-myp2H-MYO1T*). Cells were grown and mounted in CSM-Trp$^-$ medium. (C) In a separate experiment budding yeast cells (as in B and F) were grown in CSM-Trp$^-$ medium and scored for cytokinesis defects measured as the fraction of cells forming connected cell chains, as described by Tolliday *et al*. (2003). (D) DIC (left) and fluorescence (right) micrographs of budding yeast *myo1*Δ *she4*Δ cells expressing GFP-Myo1p (*pGFP-MYO1*), GFP (*pPGT*), or GFP-Myo2H-Myo1Tp (*pGFP-myo2H-MYO1T*). (E) In a separate experiment, budding yeast cells (as in D) were grown in CSM-Trp$^-$ medium and scored for cytokinesis defects. (F) DIC (left) and fluorescence (right) micrographs of budding yeast *myo1*Δ cells expressing GFP-Myo1Tp (Myo1p tail). Bars, 5 μm.

GFP fusion proteins/stains were imaged in cells as described previously (Lord and Pollard, 2004). Images were captured with an Olympus IX71 microscope by epifluorescence illumination with a PlanApo 60× (1.4 numerical aperture) objective and recorded with an Orca-ER cooled charge-coupled device camera (Hamamatsu, Bridgewater, NJ). For spinning disk confocal microscopy, the microscope was connected to a confocal scanner (model UltraView RS; PerkinElmer Life and Analytical Sciences, Boston, MA), and the images were captured with a PlanApo 100× (1.4 numerical aperture) objective. Images were acquired and processed using MetaMorph, Utra View RS, Image J, and PhotoShop software.

## *Plasmid Construction*

Table 2 lists the plasmid constructs used in this study. The fidelity of constructs was verified by automated DNA sequencing (Keck Facility, Yale University, New Haven, CT).

***pPGT.*** An 800-base pair region upstream of the *MYO1* open reading frame (ORF) (encompassing the *MYO1* promoter) was amplified from budding yeast genomic DNA with 5′ *KpnI*-prom (GGTACCGCATAGACGATCTCTACGAC) and 3′ *XhoI*-prom (CTCGAGTATTGCTGTTGTTGTCCTGTC). The DNA was ligated into *KpnI/XhoI* linearized *pRS314.* A *XhoI/NotI* GFP fragment was liberated from *p573–81X* and ligated into *XhoI/NotI* linearized *pRS314-MYO1promoter* plasmid. A 1-kb terminator region immediately downstream of the *MYO1* ORF was amplified from genomic DNA with 5′ *NotI-SalI*-term (GCGGCCGCTTTAGTCGACGACGACACGAGCGTTATATAC) and 3′ *SacI*-term (GAGCTCGAATTATTCCACATCCAGATCGG) and ligated into *NotI/SacI* linearized *pRS314-MYO1promoter-GFP* to yield *pPGT*.

***pGFP-myo2/-myp2.*** *NotI/SalI myo2* and *myp2* fragments were liberated from *p573–81X-myo2* and *p573–81X-myp2*, respectively, and ligated into *NotI/SalI* linearized *pPGT*, generating *pGFP-myo2* and *pGFP-myp2*.

***pGFP-MYO1/MYO1H-myo2T/-MYO1H-myp2T/-myo2H-MYO1T/-myp2H-MYO1T.*** We constructed chimeric myosin head-tail budding yeast constructs using a cloning strategy similar to that used to generate fission yeast myosin-II chimeras (Bezanilla and Pollard, 2000). As with *myo2* and *myp2*, the conserved sequence CCTTGG (encoding amino acids ProTrp in each myosin-II) was found at the junction of the DNA regions encoding the head and tail of Myo1p (base pairs 2524–2529, aa 842–843). When constructing the 3′ head and 5′ tail primers for *MYO1*, we took advantage of this sequence by making a silent point mutation (CCTTGG to CCATGG) to engineer an *NcoI* site (Figure 1A). The *MYO1* head and tail encoding fragments were amplified from *YCp50-MYO1* using the following primers: 5′ *NotI-MYO1* (GCGGCCGCATGACCGGCGGGCAGTCTTGC) and 3′ *SalI-NcoI-MYO1* head (ACGCGTCGACGTCAAACCATGGATCTTCCTTCACCAGTC); 5′ *NcoI-MYO1* tail (GACTGGTGAAGGAAGATCCATGGTTTA) and 3′ *SalI-MYO1* (GTCGACCGTTAACTGAAAATTTTACTCTGTGC). Two naturally occurring *NcoI* sites are present within the *GFP-MYO1* head sequence (one in the *GFP* ORF and one in the *MYO1* head sequence). To overcome this complication, we used the following cloning strategy: *pPGT* was linearized with *NcoI* and *SalI*, and an *NcoI/SalI MYO1* tail fragment was subsequently inserted to yield a construct carrying a 5′ portion of *GFP* fused to the *MYO1* tail sequence. In parallel, a *NotI/SalI-NcoI MYO1* head fragment was inserted into *NotI/SalI* linearized *pPGT*. This construct was in turn subjected to partial *NcoI* digestion to liberate a 3′ portion of *GFP* fused to the complete *MYO1* head sequence (i.e., with its naturally occurring, internal *NcoI* site intact). This fragment ("3kb *MYO1* head") was subsequently inserted into the plasmid carrying part of *GFP* fused to the *MYO1* tail (see above) via the *NcoI* site to yield *pGFP-MYO1*. Correct orientation of the 3kb *MYO1* head fragment was tested using restriction digest analysis. The modular head-tail GFP-*MYO1* construct was fully functional as judged by its ability to complement *myo1*Δ strains. Having generated a modular head-tail construct compatible (in terms of reading frame, and the presence of an *NcoI* site at the head-tail junction) with *myo2* and *myp2* chimeric modular constructs (*p81myo2H-myp2T* and *p81-myp2H-myo2T*), chimera constructs (*pGFP-MYO1H-myo2T/-MYO1H-myp2T/-myo2H-MYO1T/-myp2H-MYO1T*) were generated by replacing *MYO1* head and tail modules using appropriate *NcoI* GFP-head and *NcoI/SalI* tail fragments (as detailed above for *pGFP-MYO1*). Regular digests (not partial digests) were used to liberate *GFP-myo2/myp2* 3kb head fragments because, unlike *MYO1*, neither *myo2* or *myp2* possesses naturally occurring *NcoI* sites.

***pGFP-MYO1T.*** The GFP-Myo1p tail fusion was constructed by replacing the *NcoI GFP-MYO1* head fragment (3kb *MYO1* head) in *pGFP-MYO1H-MYO1T* with an *NcoI GFP* fragment generated from a GFP sequence amplified from *p573–81X* with primers: 5′ *XhoI*-GFP (CTCGAGATGTCTTTGAGTAAAGGAGAAGAACTTTTCAC) and 3′ *NcoI*-GFP (CCATGGTTTGTATAGTTCATCCATGCCATG). Correct orientation of *GFP* was tested using restriction digests.

***p81-MYO1H-myo2T/-MYO1H-myp2T/-myo2H-MYO1T/-myp2H-MYO1T.*** Fission yeast *MYO1-myo2/myp2* chimera plasmids were constructed using head-tail module replacement (as described for budding yeast *pPGT* chimera plasmids; see above). *p81-myo2H-myp2T* and *p81-myp2H-myo2T* plasmids were linearized appropriately and used to generate the various chimera constructs by religation with appropriate modules.

**Table 3.** Functionality of myosin-II chimeras and truncations

| | Tail | | | |
|---|---|---|---|---|
| Head | Myo1p | Myo2p | Myp2p | None |
| Fulfils Myo1p function/contractile ring localization in budding yeast (*myo1*Δ[a]) | | | | |
| Myo1p | Yes/yes | No/no | No/no | ND |
| Myo2p | Yes/yes | No/no | ND | ND |
| Myp2p | Yes/yes | ND | No/no | ND |
| None | Yes/yes | ND | ND | No/no |
| Fulfils Myo2p function/contractile ring localization in fission yeast (*myo2-E1*) | | | | |
| Myo1p | No/no | No/yes | ND | ND |
| Myo2p | No/yes | Yes/yes | No/yes[b] | No/yes |
| Myp2p | ND | Yes/yes[b] | No/yes[b] | ND |
| None | ND | No/no[c] | ND | No/no |
| Fulfils Myp2p function/contractile ring localization in fission yeast (*myp2*Δ*)* | | | | |
| Myo1p | No/no | ND | No/no | ND |
| Myo2p | ND | No/yes[b] | Yes/yes[b] | ND |
| Myp2p | No/yes | No/yes[b] | Yes/yes | No/no[d] |
| None | ND | ND | No/no[d] | No/no |

ND, no data.

[a] *myo1*Δ in BF264-Du background (JC 1609).

[b] Bezanilla and Pollard, 2000.

[c] Naqvi *et al.* (1999).

[d] Data from GFP-fusions constructed by chromosomal integration of GFP into fission yeast (FY 527).

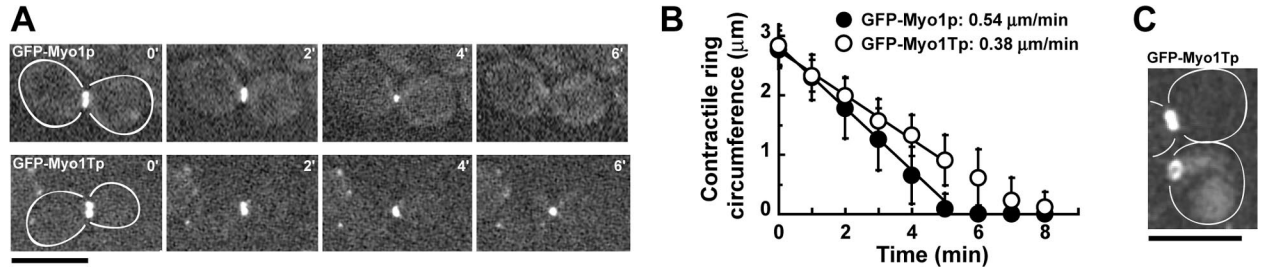


**Figure 2.** Dynamics of the Myo1p tail contractile rings are similar to those of full-length Myo1p contractile rings. (A) Time-lapse spinning disk confocal fluorescence micrographs of *myo1*Δ strain (BF264-Du) cells expressing GFP-Myo1p (top) or GFP-Myo1Tp (bottom) during contractile ring constriction. Stacks of six confocal z sections at intervals of 0.5 μm were collected (projected at maximum intensity) at each time point. Dividing cells are outlined in white in the first panels. (B) Plot of average contractile ring circumference versus time for 20 GFP-Myo1p cells (closed circles) and 20 GFP-Myo1Tp cells (open circles). Time zero represents the point at which rings initiate contraction. The slopes of these plots are the rates of ring constriction. (C) Confocal fluorescence micrograph of two cells carrying the GFP-Myo1Tp construct (as described in A). The lower cell is orientated such that a complete, uninterrupted contractile ring is apparent. Bars, 5 μm.

***p81-MYO1.*** A *NotI/SalI MYO1* fragment was liberated from *pGFP-MYO1* and ligated into *NotI/SalI* linearized *p573–81X*.

***p81-myo2H.*** A subfragment-1 (S1)-like Myo2Hp-GFP ("S1-like") head fusion was constructed by inserting a *myo2* head fragment into *p572–81X*. The head (base pairs 1–2445; aa 1–815) was amplified using the primers 5′ *NotI-myo2-S1* (GCGGCCGCGGCGGTGGAATGACAGAAGTAATATCTAATAAAATAACTGC) and 3′ *NotI-myo2-S1* (GCGGCCGCCGGGCCTTAGATTGAAAAATAACTTAGC).

***pFA6a-natMX4.*** This plasmid was generated by replacing the *NcoI/SacI kan*$^R$ fragment from *pFA6a-kanMX6* (Bahler *et al*., 1998) with an *NcoI/SacI nat*$^R$ fragment derived from pAG36 (Goldstein and McCusker, 1999).

### *Database Searches and Sequence Analysis*

Uncharacterized fungal myosin-II amino acid sequences were identified from various genomes via sequence homology comparisons with the *S. pombe* Myo2p motor. The following resources were used: *Aspergillus fumigatus* Genome Project (http://www.tigr.org/tdb/e2k1/afu1/), Munich Information Center for Protein Sequences (http://mips.gsf.de/projects/fungi/neurospora.-html), *Candida albicans* Research Lab (http://candida.bri.nrc.ca/candida/index.cfm), and *Cryptococcus neoformans* Genome Project (http://www.tigr.org/tdb/e2k1/cna1/). The probability of coiled-coil conformation within myosin-II tail sequences was calculated by the COILS program (Lupas *et al*., 1991; http://www.ch.embnet.org/software/COILS_form.html) using a window width of 28 amino acids.

## RESULTS

### *Budding Yeast Myo1p Functions without Its Head*

Initial studies using myosin-II chimeras (consisting of various combinations of Myo1p, Myo2p, and Myp2p heads and tails) led to a surprising observation. Like full-length GFP-Myo1p, chimeras consisting of either the GFP-Myo2p head or the GFP-Myp2p head fused to the Myo1p tail localized to contractile rings and rescued cytokinesis defects of the budding yeast *myo1*Δ strain (Figure 1B and Table 3). Like GFP-Myo1p (Figure 1B), expression of either GFP-Myo2H-Myo1Tp or GFP-Myp2H-Myo1Tp fully overcame the undivided, multinucleate phenotype of *myo1*Δ cells (Figure 1C). The finding was surprising, because Myo2p motor function and fission yeast cytokinesis require the UCS protein Rng3p (Wong *et al*., 2000; Lord and Pollard, 2004). Naturally, Rng3p is not present in budding yeast, and She4p, the only UCS protein common to budding yeast, is specific for type I and V myosins, not type II Myo1p (Toi *et al*., 2003; Wesche *et al*., 2003). To rule out the possibility that She4p was substituting for Rng3p in budding yeast to promote the motor activity of the GFP-Myo2H-Myo1Tp, we tested the ability of this chimera to rescue loss of Myo1p function in a double deletion mutant lacking both *MYO1* and *SHE4*. In the absence of any UCS protein, GFP-Myo2H-Myo1Tp still fully supported Myo1p function (Figure 1, D and E).

To test whether the motor domain is required for myosin-II function in budding yeast, we constructed a truncated form of Myo1p consisting of GFP fused to the Myo1p tail. Remarkably, plasmid expression of this headless Myo1p construct (GFP-Myo1Tp) fully restores Myo1p function in a *myo1*Δ strain (BF264-Du) and concentrates in a ring at the

**Table 4.** Summary of complementation results for haploid segregants generated in the W303a background after plasmid transformation, sporulation, and tetrad dissection of a *myo1*Δ*/MYO1* heterozygous diploid (RLY 1236)

| | Recovered segregants | | |
|---|---|---|---|
| Plasmid | *TRP1*$^+$ [a] | *kan*$^R$ [b] | *TRP1*$^+$ *kan*$^R$ [c] |
| *pPGT (vector alone)* | 32 | 0 | 0 |
| *pGFP-MYO1* | 62 | 29 | 29 |
| *pGFP-MYO1-Tail* | 55 | 23 | 23 |

[a] *TRP1*$^+$ represents haploids carrying the indicated *TRP1*-containing plasmid from segregants generated from 25 tetrads (per transformant). Recovered segregants lacking a *TRP1*$^+$ phenotype have presumably undergone plasmid loss during sporulation and tetrad dissection onto YPDa plates.

[b] *kan*$^R$ represents haploid segregants that possess the *myo1*Δ*:kan*$^R$ deletion. Haploids lacking *kan*$^R$ resistance (geneticin-sensitive) constitute segregants possessing an intact *MYO1* gene.

[c] All *kan*$^R$ segregants analyzed were found to be complemented (*TRP1*$^+$) by either full-length GFP-Myo1p or the GFP-Myo1p tail. No *kan*$^R$ segregants were derived from *pPGT* (vector alone) transformants. These data are consistent with both GFP-Myo1p and the GFP-Myo1p tail supporting the essential function of Myo1p in this strain background (W303a) where *myo1*Δ is lethal.

bud neck (Figures 1F and 2C). Like GFP-Myo1p, expression of GFP-Myo1Tp fully overcame the undivided, multinucleate phenotype of *myo1*Δ cells (Figure 1C).

The ring of GFP-Myo1Tp in the bud neck constricted during cytokinesis (Figure 2A) at 70% the rate of *myo1*Δ cells expressing full-length Myo1p (Figure 2B). This small reduction in the rate of contraction had no effect on the ability of GFP-Myo1Tp to fully complement a *myo1*Δ strain (Figure 1, B and C), because the cell doubling time was indistinguishable from that of *myo1*Δ cells carrying GFP-Myo1p (our unpublished data). The slightly reduced rate of Myo1Tp contraction may reflect a nonessential role for the Myo1p motor in contractile ring disassembly, as proposed for Mlc2p (Luo *et al*., 2004). To confirm that the Myo1p tail contractile rings, such as those shown in Figure 2A, were actually true rings, we imaged cells orientated in the mounting medium with their emerging buds pointing toward the objective. Like full-length Myo1p (Bi *et al*., 1998), the Myo1p tail formed complete, uninterrupted rings (Figure 2C). Thus, the budding yeast contractile ring constricts competently during cytokinesis in the absence of myosin-II motor activity.

Myo1p tail alone was also sufficient for function in the W303a strain, where loss of *MYO1* is lethal. We transformed the heterozygote *myo1*Δ/*MYO1* diploid with plasmids carrying GFP (vector alone), GFP-Myo1p, or GFP-Myo1Tp. Transformants were sporulated and tetrads dissected to test which of these plasmids rescue the lethality of haploid *myo1*Δ segregants. The vector alone did not rescue, whereas both GFP-Myo1p and GFP-Myo1Tp constructs rescued *myo1*Δ lethality as demonstrated by recovery of $kan^R$ segregants complemented ($Trp^+$) by *pPGT-MYO1* or *pPGT-MYO1T* (Table 4). Both GFP-Myo1p and GFP-Myo1Tp concentrated in contractile rings at mother-bud necks (Figure 3, A and B). The phenotypes of strains rescued by full-length GFP-Myo1p and GFP-Myo1Tp were phenotypically identical (Figure 3, A–C).

### *Fission Yeast Myosin-II Function Requires Both Heads and Tails*

In contrast to budding yeast Myo1p, both the head and tail of fission yeast Myp2p are essential for function. Unlike full-length GFP-Myp2p, tailless (GFP-Myp2Hp) or headless (GFP-Myp2Tp) forms of Myp2p did not support *myp2* function (Figure 4, A and B, and Table 3). Cells expressing truncated forms of Myp2p from integrated *GFP-myp2 head* or *GFP-myp2 tail* gene fusions displayed cytokinesis defects similar to a *myp2*Δ strain (Figure 4, A and B). Both Myp2p tailless and headless GFP fusions failed to concentrate in contractile rings (Figure 4C and Table 1C). GFP-Myp2Tp formed punctate aggregates, whereas GFP-Myp2Hp exhibited a diffuse cytoplasmic localization pattern, very much unlike the distribution of full-length GFP-Myp2p (Figure 4C).

Previous work established that Myo2p function requires both its catalytic head and tail domains. Point mutations in either the Myo2p head (Naqvi *et al*., 1999) or tail (Mulvihill *et al*., 2001; Motegi *et al*., 2004) can compromise function. Interestingly, a Myo2p Head-GFP fusion localized at contractile rings in wild-type or *myo2-E1* cells, despite being unable to substitute functionally for *myo2* (Table 3 and Figure 4D). Presumably, the Myo2p motor alone has a higher affinity for contractile ring actin filaments than the Myp2p motor domain. Expression of the Myo2p tail in *myo2*Δ strains fails to support contractile ring formation and cell division (Naqvi *et al*., 1999; Table 3) in spite of diffuse accumulation of the Myo2p tail at the medial division site.

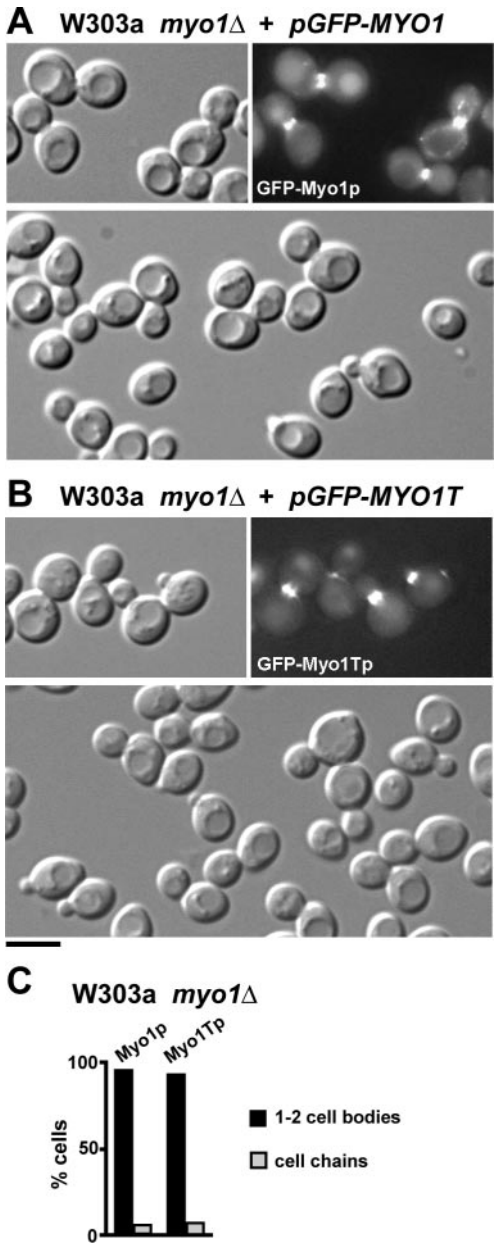


**Figure 3.** The Myo1p tail alone provides the full function of Myo1p during cytokinesis and cell growth in the W303a background. DIC (top left and bottom) and fluorescence (top right) micrographs of W303a budding yeast *myo1*Δ haploids expressing GFP-Myo1p (*pGFP-MYO1*) (A) and GFP-Myo1Tp (*pGFP-MYO1T*) (B). (C) In a separate experiment budding yeast cells (as in A and B) were grown in CSM-$Trp^-$ medium and scored for cytokinesis defects by quantitating the percent of cells forming connected cell chains. Bar, 5 μm.

Table 3 summarizes complementation and localization experiments for myosin-II head or tail truncations and all possible combinations of Myo1p/Myo2p/Myp2p head-tail chimeras. Both the negative and positive results are consistent with previous work (Naqvi *et al*., 1999; Bezanilla and Pollard, 2000) and the interpretations made in our present study. We do not emphasize the negative results, because we cannot rule out the possibility that negative complemen-

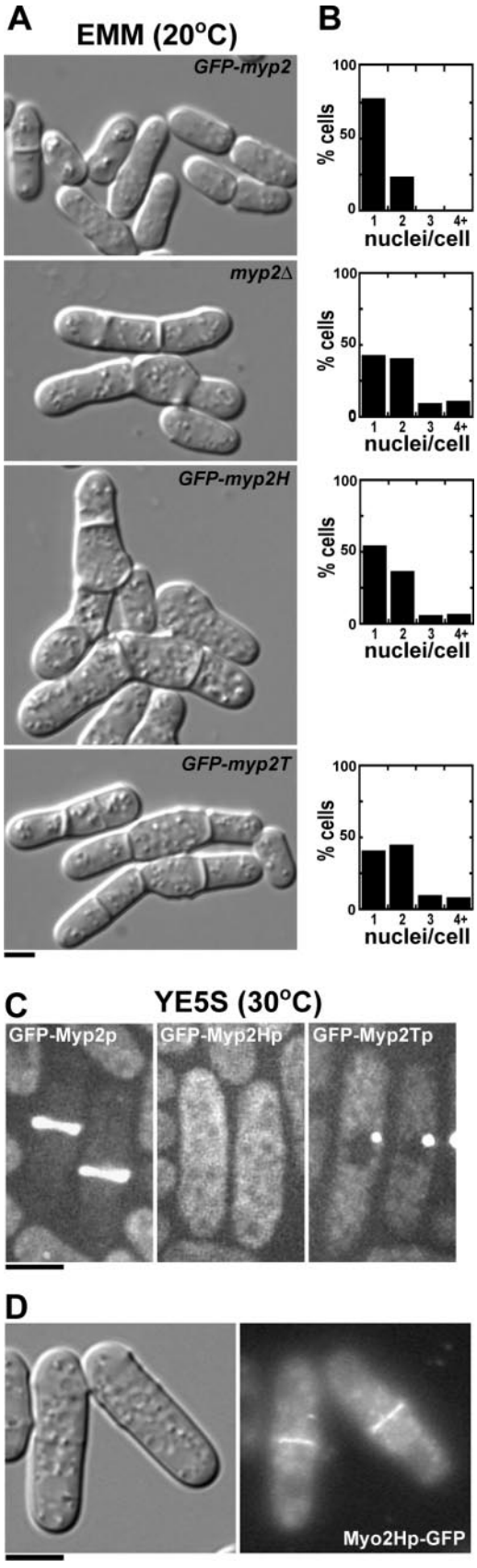


**Figure 4.** The head and tail of fission yeast Myp2p are required for its function. (A) DIC micrographs show the cell morphology for a fission yeast strain carrying a chromosomal *GFP-myp2* fusion (JW 994), a *myp2*Δ strain (TP5), and strains with chromosomal *GFP-myp2* head (EL 102) or *GFP-myp2* tail (EL 101) fusions replacing the *myp2* gene. Strains were grown under restrictive conditions for testing Myp2p function (EMM medium, 20°C). (B) In a separate experiment, cells were stained with Hoescht dye and scored for the number of nuclei per cell. (C) Fluorescence micrographs of GFP-Myp2p (JW 994, left), GFP-Myp2Hp (EL 102, center), and GFP-Myp2Tp (EL 101, right). Cells are shown after growth under permissive conditions (YE5S, 30°C). (D) DIC (left) and fluorescence (right) micrographs for a strain (FY 527) bearing *p81-myo2H* encoding the Myo2Hp-GFP fusion. Cells are shown after growth in EMM Ura$^-$ medium. Bars, 5 μm.

tation/localization results arose from inefficient expression rather than failure to function.

## DISCUSSION

In the contractile ring model of cytokinesis, actin filaments and myosin-II constrict the cleavage furrow. Presumably, the tails of the myosin-II molecules assemble bipolar filaments so that myosin heads can interact with oppositely polarized ring actin filaments in a sliding filament mechanism similar to muscle contraction. However, this and other recent work suggest that the role of myosin-II tails in cytokinesis is more complex.

Remarkably, the tail of Myo1p is essential for cytokinesis in budding yeast, but the heads are dispensable. Both the heads and tails of myosin-II are required for cytokinesis in other cells, including fission yeast. Other myosins can fulfill some of their roles in cells with compromised motor activity, but Myo1p is the first example of a myosin that functions in the complete absence of its motor domain. Mutant forms of fission yeast Myo2p possessing only 5% of the wild-type in vitro motility activity (measured by actin filament gliding assays) support normal cytokinesis in vivo (Lord and Pollard, 2004). Furthermore, mutant forms of the essential type-I myosin from *Aspergillus* with only 1% of the wild-type actin-activated ATPase activity still support normal cell growth with only minor phenotypic defects (Liu *et al*., 2001). On the other hand, *Dictyostelium* myosin-II lacking its ELC binds actin filaments but has <10% of wild-type actin-activated ATPase activity (Chen *et al*., 1995). Cells lacking ELC fail to complete cytokinesis, yet still move reasonably normally (Chen *et al*., 1995; Xu *et al*., 2001; Laevsky and Knecht, 2003).

Why should the requirements for myosin-II in cytokinesis differ in budding yeast? Two possibilities come to mind. First, contractile force may not be required for cytokinesis in budding yeast. Growth of the septum might conceivably fill in the narrow neck between mother and bud, with the ring of actin filaments growing smaller by disassembly. Alternatively, another myosin, such as *Sc* Myo2p, may interact with actin filaments to constrict the ring. Budding yeast Myo2p is an essential type-V myosin that localizes to polarized growth sites and the bud neck (Johnston *et al*., 1991; Brockerhoff *et al*., 1994; Lillie and Brown, 1994; Govindan *et al*., 1995). Vesicle targeting dependent on *Sc* Myo2p facilitates septum closure (Wagner *et al*., 2002), which may produce the force to drive ring constriction in the absence of myosin-II motor activity. A role for this type-V myosin in cytokinesis is supported by genetic studies revealing that defects of a *myo1*Δ and a *myo2-66* mutation are additive when combined (Lillie and Brown, 1998).

Despite lacking a motor domain the Myo1p tail concentrated in the ring structure at the bud neck and constricted

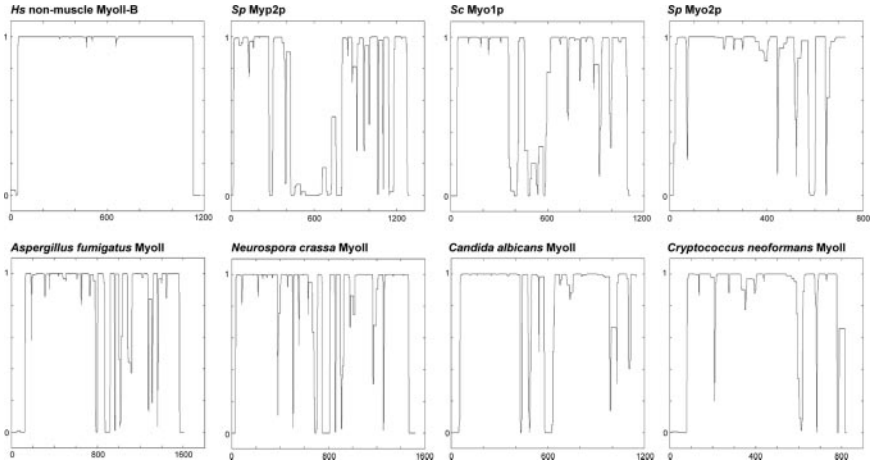


**Figure 5.** Coiled-coil predictions for different myosin-II tails. Predictions were calculated using the COILS program (Lupas *et al*., 1991). The tails of *Homo sapiens* nonmuscle myosin-II-B, *S. pombe* Myp2p, *S. cerevisiae* Myo1p, *S. pombe* Myo2p, and four uncharacterized fungal type-II myosins (from *A. fumigatus*, *N. crassa*, *C. albicans*, and *C. neoformans*) were compared. *x*-axis, amino acid position. *y*-axis, probability of forming a coiled-coil.

with almost wild-type dynamics. Tolliday *et al*. (2003) reported an unpublished observation that a construct consisting of the C-terminal 868 amino acids of Myo1p localizes to the bud neck. However, overexpression of this C-terminal region of Myo1p from the *GAL1* promoter was toxic resulting in cells with cytokinesis defects reminiscent of a *myo1*Δ strain (Tolliday *et al*., 2003). This phenotype presumably stems from complications caused by an overabundance of the Myo1p tail, because we find that expression of the Myo1p tail from its native promoter had no negative effects on cytokinesis.

Because the function of budding yeast myosin-II does not depend on its motor domain, its tail must contribute productively to cytokinesis in some way. Myo1p tails might form filaments or interact with septins and/or other structural proteins to stabilize the bud neck during bud outgrowth, allowing other mechanisms such as new membrane addition and septum formation to close the narrow orifice. Indeed, ring contraction and primary septum formation are interdependent (Schimdt *et al*., 2002; VerPlank and Li, 2005), suggesting a role for Myo1p in coordinating septation and contractile ring closure. Myosin-II contributes to the mechanical stability of the cortex in *Dictyostelium* (Pasternak *et al*., 1989), although this is thought to depend on interaction of the heads with actin filaments.

Assembly of contractile rings also differs in the two yeast. Budding yeast Myo1p occurs at the division site much earlier in the cell cycle ($G_1$/S) than in other cells. Further Myo1p localization depends on assembly of the septin complex (Bi *et al*., 1998; Lippincott and Li, 1998). Fission yeast septins are not essential and occur at the division site later than Myo2p and Myp2p (Wu *et al*., 2003; An *et al*., 2004).

### *Features of the Myo1p Tail That May Support a Novel Role for Myosin-II in Fungal Cytokinesis*

Available genome sequences show that fungi have two types of myosin-II. Some, including *S. pombe* Myo2p and *Cryptococcus neoformans* MyoII, have tails like conventional animal myosin-II, consisting almost entirely a coiled-coil. Most sequenced fungal myosin-II molecules, including *S. pombe* Myo1p and *S. cerevisiae* Myp2p, have tails with two distinct regions of predicted coiled-coil separated by a nonhelical insert of 200–270 amino acids (Bezanilla *et al*., 1997; Figure 5). Other examples include MyoII from *Aspergillus fumigatus*, MyoII from *Candida albicans*, and MyoII from *Neurospora crassa*. These inserts of 200 or more residues include 5–10% prolines, which are rarely found in the coiled-coil tails of conventional myosin-II. Hydrodynamic analysis established that the isolated Myo2p tail forms conventional dimers, whereas the Myp2p tail is monomeric, folding back on itself to form an antiparallel coiled-coil (Bezanilla and Pollard, 2000). Although Myo1p and Myp2p are not functional homologues, the ability of the Myo1p tail to support cytokinesis may arise from an antiparallel coiled-coil structure similar to Myp2p. Given this novel structure, approaches other than simple truncations will be required to determine how the tail of Myo1p functions in cytokinesis without its heads.

### *Fission Yeast Type-II Myosins Function as Actin-dependent Motors*

Unlike budding yeast, fission yeast type-II myosins act like other conventional myosins, requiring both their heads and tails for function. Point mutations in either the head or tail of Myo2p can attenuate its function (Balasubramanian *et al*., 1998; Naqvi *et al*., 1999; Wong *et al*., 2000; Motegi *et al*., 2004), whereas truncated forms of Myp2p lacking either the head or tail fail to support Myp2p function. Second, unlike Myo1p, both Myo2p and Myp2p require actin filaments to form a compact contractile ring at anaphase A (Naqvi *et al*., 1999; Wu *et al*., 2003). Third, Myo2p contractile ring localization requires Rng3p, a motor regulator (Wong *et al*., 2000; Lord and Pollard, 2004), whereas the Rng3p homolog (She4p) is dispensable for the localization and function of Myo1p (Toi *et al*., 2003; Wesche *et al*., 2003). Fourth, loss of the *S. pombe* RLC (Rlc1p) results in temperature-sensitive lethality (Le Goff *et al*., 2000; Naqvi *et al*., 2000), whereas loss of the budding yeast RLC (Mlc2p) has only very subtle effects on Myo1p localization and cytokinesis (Luo *et al*., 2004). As in cells possessing headless Myo1p, deletion of *MLC2* yields Myo1p contractile rings that disappear slower than in wild-type strains, suggesting a nonessential role for Mlc2p and Myo1p motor activity in ring disassembly (Luo *et al*., 2004). Such subtle effects on contractile ring dynamics suggest that the Myo1p motor may play a secondary, nonessential role in budding yeast cytokinesis. The fact that Myo2p and Myp2p motor activity is essential for myosin-II function represents an advantage for fission yeast in studying cytokinesis powered by conventional myosin-II behavior.

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