

# Current Biology

## Motor Activity Dependent and Independent Functions of Myosin II Contribute to Actomyosin Ring Assembly and Contraction in *Schizosaccharomyces pombe*

### Highlights

- In many eukaryotes, cytokinesis requires an actomyosin-based contractile ring
- The role of motor activity of myosin II in cytokinesis is a topic of active debate
- We isolate a new allele of *S. pombe* Myo2, an essential myosin heavy chain
- We show motor activity-dependent and -independent roles for Myo2

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### In Brief

Cytokinesis in many eukaryotes requires an actomyosin-based contractile ring. The role of the motor protein Myosin II in cytokinesis is actively debated. Palani et al. identify a new motor activity-defective allele of fission yeast myosin II and report that the motor activity is dispensable for ring assembly but is essential for ring contraction.



# Motor Activity Dependent and Independent Functions of Myosin II Contribute to Actomyosin Ring Assembly and Contraction in *Schizosaccharomyces pombe*

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## SUMMARY

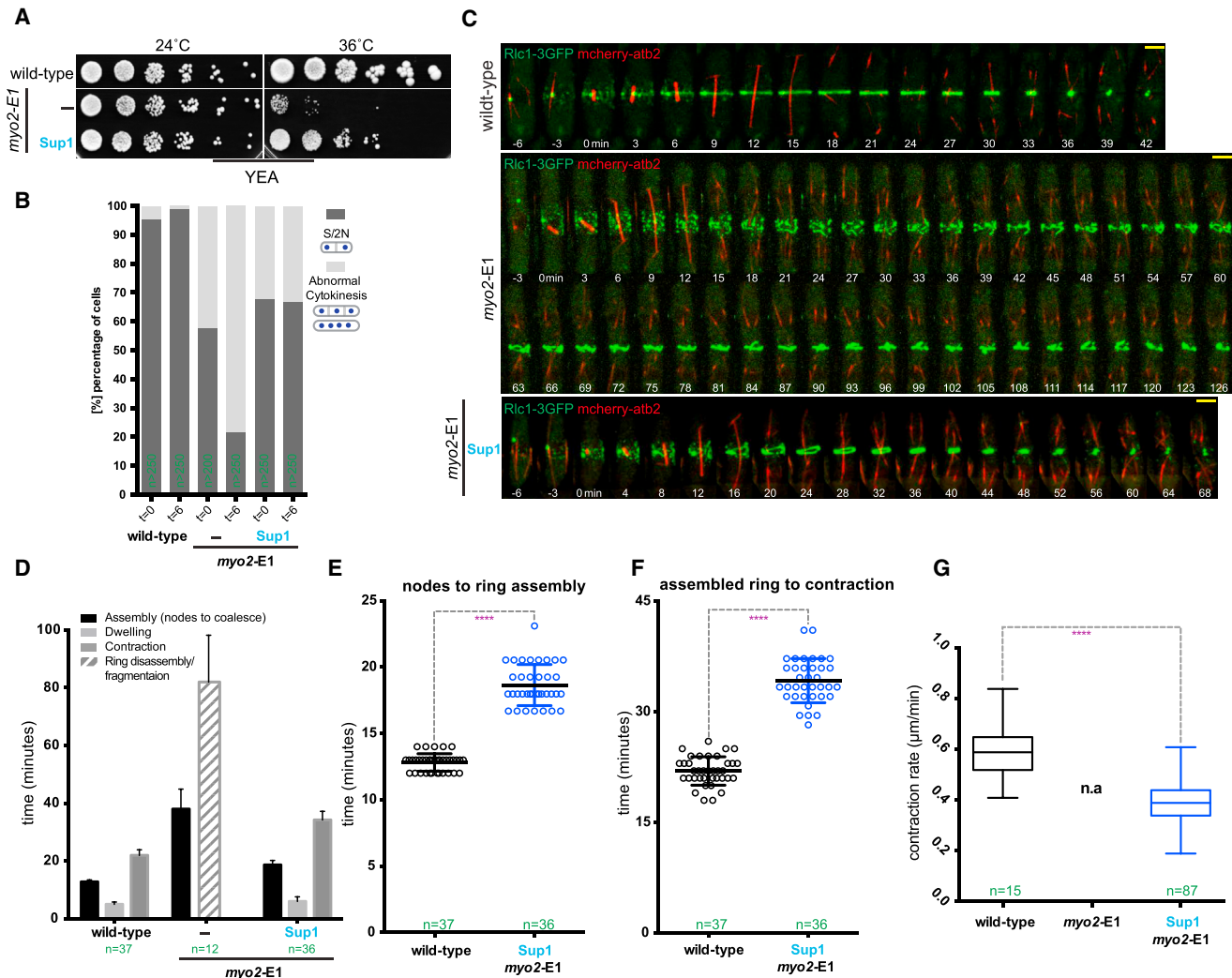
Cytokinesis depends on a contractile actomyosin ring in many eukaryotes [1–3]. Myosin II is a key component of the actomyosin ring, although whether it functions as a motor or as an actin cross-linker to exert its essential role is disputed [1, 4, 5]. In *Schizosaccharomyces pombe*, the *myo2-E1* mutation affects the upper 50 kDa sub-domain of the myosin II heavy chain, and cells carrying this lethal mutation are defective in actomyosin ring assembly at the non-permissive temperature [6, 7]. *myo2-E1* also affects actomyosin ring contraction when rings isolated from permissive temperature-grown cells are incubated with ATP [8]. Here we report isolation of a compensatory suppressor mutation in the lower 50 kDa sub-domain (*myo2-E1-Sup1*) that reverses the inability of *myo2-E1* to form colonies at the restrictive temperature. *myo2-E1-Sup1* is capable of assembling normal actomyosin rings, although rings isolated from *myo2-E1-Sup1* are defective in ATP-dependent contraction in vitro. Furthermore, the product of *myo2-E1-Sup1* does not translocate actin filaments in motility assays in vitro. Superimposition of *myo2-E1* and *myo2-E1-Sup1* on available rigor and blebbistatin-bound myosin II structures suggests that *myo2-E1-Sup1* may represent a novel actin translocation-defective allele. Actomyosin ring contraction and viability of *myo2-E1-Sup1* cells depend on the late cytokinetic *S. pombe* myosin II isoform, *Myp2p*, a non-essential protein that is normally dispensable for actomyosin ring assembly and contraction. Our work reveals that *Myp2p* may function in two different and essential modes during cytokinesis: a motor activity-independent form that can promote actomyosin

ring assembly and a motor activity-dependent form that supports ring contraction.

## RESULTS AND DISCUSSION

The product of the *myo2-E1* allele is predicted to harbor a substitution of glycine at position 345 with arginine (Figures S1A and S1B). Cells carrying this mutant allele are capable of colony formation at 24°C but are severely compromised for colony formation at 36°C (Figure 1A) due to defective actomyosin ring assembly [6, 7, 9, 10]. The *myo2-E1* mutation resides between  $\alpha$ -helix HL and  $\beta$  sheet S1D, which is part of the upper 50 kDa sub-domain in the head of *Myo2p* (Figure S1B). Previous work has shown that *Myo2-E1p* (product of *myo2-E1*) does not bind or move actin filaments and has a very low ATPase activity in vitro [10, 11]. The presence of a bulky arginine side chain between helices HL and HO in the upper 50 kDa sub-domain of this mutant might introduce constraints to the conformational changes in the *Myo2p* head domain during the actomyosin cycle, resulting in the observed phenotypes. To further understand the role of *Myo2p* in cytokinesis, we isolated genetic suppressors that restored the ability of *myo2-E1* cells to form colonies at 36°C (Figure 1A). One suppressor, *myo2-E1-Sup1*, is described in this study. Genetic crosses between *myo2-E1-Sup1* and wild-type cells only produced progeny that were able to form colonies at 36°C, suggesting that the suppressor mutation was intragenic or very tightly linked to *myo2*. Nucleotide sequence determination revealed that *myo2-E1-Sup1* contained the original G345R mutation and also had additional mutations (Q640H and F641I) (Figures S1A and S1B). Furthermore, no sequence alterations were found in the neighboring *rgf3* gene (data not shown), which has also been implicated in cytokinesis [12, 13]. Therefore, we concluded that the sequence alteration Q640H F641I was responsible for the suppression of *myo2-E1*. Interestingly, Q640H and F641I are located in the HW region of the *Myo2p* head (within the lower 50 kDa sub-domain), which is at a significant distance (~36 Å) from HL and S1D, the region where the original mutation resides, suggesting





### Figure 1. *myo2-E1-Sup1* Restores Actomyosin Ring Assembly and Partial Ring Contraction

(A) Serial dilutions (10-fold) of wild-type, *myo2-E1*, and the intragenic suppressor *myo2-E1-Sup1* were spotted onto yeast extract agar (YEA) plates and grown for 3 days at 24°C and 36°C.

(B) Quantification of DAPI and anillin blue staining used to visualize the nucleus and septum of wild-type, *myo2-E1*, and *myo2-E1-Sup1* cells, respectively. Phenotypes of the mutants were categorized into two types: septa with two nuclei (S/2N) and cells with abnormal cytokinesis, revealed by the presence of multiple septa and nuclei (MS/>2N).

(C) Time-lapse series of wild-type, *myo2-E1*, and *myo2-E1-Sup1* cells expressing 3GFP-tagged myosin regulatory light chain (Rlc1-3GFP) as a contractile ring marker and mCherry-tagged tubulin (mCherry-atb2) as a cell-cycle stage marker. Cells were grown at 24°C and shifted to 36°C for 3–4 hr before imaging at 36°C (t = 0 indicates the time before Rlc1-3GFP nodes localize to the cell middle). Images shown are maximum-intensity projections of z stacks. Scale bars represent 3 μm.

(D) Timing of contractile ring assembly, maturation/dwelling, and contraction. Quantification of (C) is shown. Error bars represent SD.

(E) Timing of actomyosin ring assembly from nodes. Quantification of (C) is shown (asterisks indicate the statistical significance of the difference between the two genotypes). Statistical significance was calculated by Student's t test (\*\*\*\*p < 0.0001). Error bars represent SD.

(F) Timing of actomyosin ring contraction. Quantification of (C) is shown. Statistical significance was calculated by Student's t test (\*\*\*\*p < 0.0001). Error bars represent SD.

(G) Constriction rate determined from a graph of ring circumference versus time. Statistical significance was calculated by Student's t test (\*\*\*\*p < 0.0001). Error bars represent SD.

See also [Figure S1](#).

potential allosteric mechanisms, rather than a simple reversal of original mutation, may operate in the suppression.

Following a 6 hr shift to 36°C, nearly 80% of *myo2-E1* cells became multinucleate and had either improper septa with a wavy and patchy appearance or did not have a septum (Fig-

ure 1B). By contrast, only ~35% of *myo2-E1-Sup1* cells contained such defects, while those defects were rarely seen in wild-type cells (Figures 1B and S1C). Since the ingressing actomyosin ring guides division septum assembly, we investigated the dynamics of the actomyosin ring component Rlc1p-3GFP

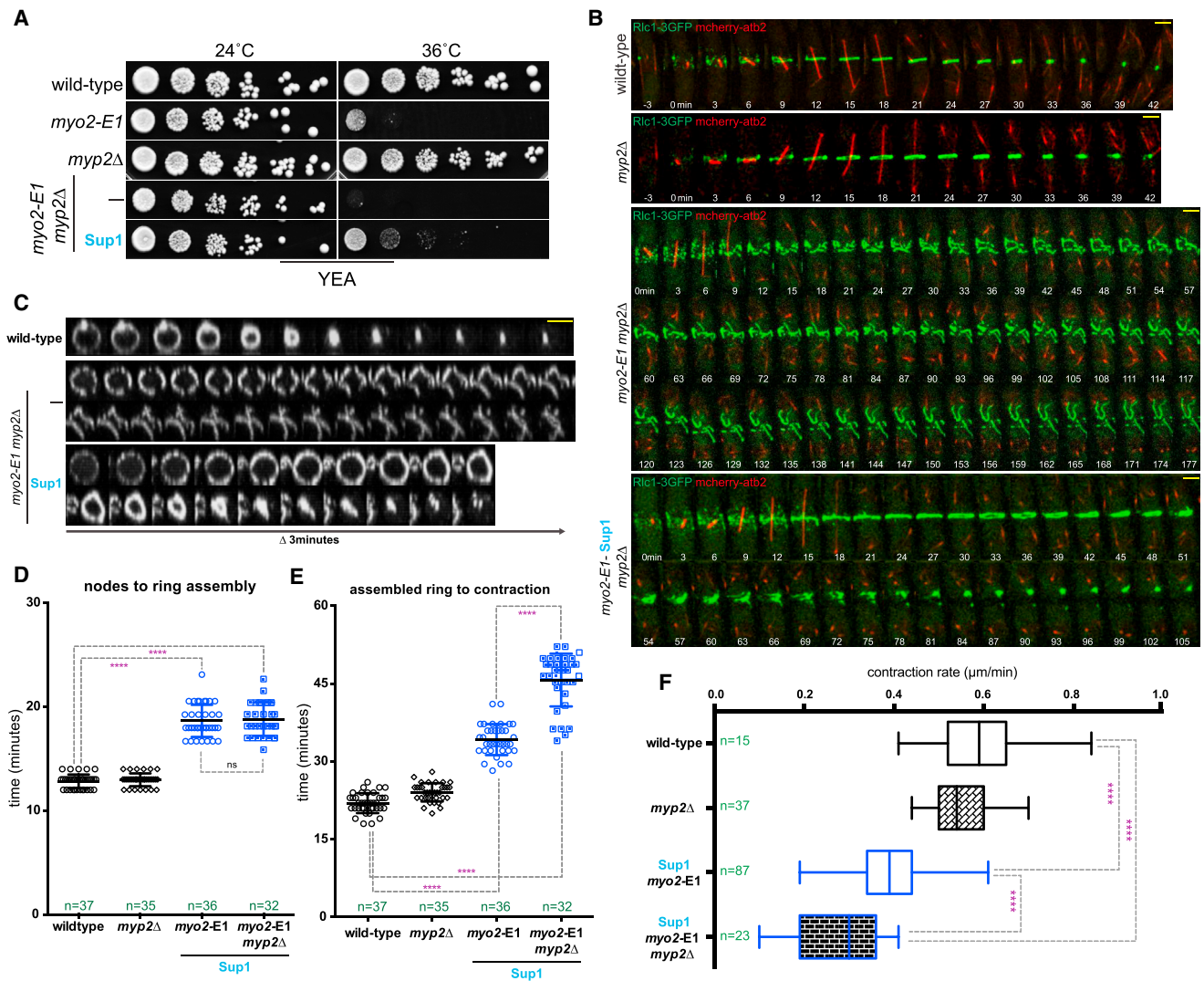
in wild-type, *myo2-E1*, and *myo2-E1-Sup1* strains; mCherry-tubulin served as a cell-cycle marker in these experiments. In wild-type cells, actomyosin rings were assembled in metaphase/anaphase A in  $\sim 12.8 \pm 0.6$  min and contracted following spindle breakdown in  $\sim 22 \pm 1.9$  min, with an intervening dwell phase of  $5 \pm 0.8$  min during which the actomyosin ring was stably maintained (Figures 1C–1E and S1D). As expected, all aspects of cytokinesis were slower in *myo2-E1* mutants compared to wild-type cells: improper ring assembly took  $\sim 38 \pm 6.9$  min and improper contraction/disassembly lasted  $\sim 82 \pm 16.2$  min at  $36^\circ\text{C}$  (Figures 1C and S1D). Imaging *myo2-E1-Sup1* cells revealed that they assembled actomyosin rings of normal appearance (Figure 1C, time point 24 min, ending on views in Figure S1D), with a significantly accelerated kinetics for both ring assembly ( $\sim 18.6 \pm 1.5$  min) and contraction ( $\sim 34.2 \pm 3$  min) compared to the original *myo2-E1* mutant. Nevertheless, both steps were marginally slower in *myo2-E1-Sup1* compared to wild-type cells (Figures 1C–1E and S1D). Whereas actomyosin rings in wild-type cells contracted at  $\sim 0.6 \pm 0.1$   $\mu\text{m}/\text{min}$ , contraction rate in *myo2-E1-Sup1* cells was  $\sim 0.4 \pm 0.08$   $\mu\text{m}/\text{min}$  at  $36^\circ\text{C}$ . These experiments established that *myo2-E1-Sup1* assembled contractile rings of normal appearance, although both ring assembly and ring contraction took  $\sim 1.5$  times longer compared to wild-type cells.

Two type II myosin heavy chains participate in cytokinesis in *S. pombe* [14–17]. We therefore investigated the possibility that Myp2p, which is normally non-essential for ring assembly, assisted in actomyosin ring assembly and contraction in the *myo2-E1-Sup1* strain through a potential ectopic upregulation. Toward this goal, we generated a double mutant of the genotype *myo2-E1-Sup1 myp2 $\Delta$* . Although this strain was viable at  $24^\circ\text{C}$ , surprisingly, it was inviable at  $36^\circ\text{C}$  (Figure 2A). Time-lapse microscopy was performed on wild-type, *myo2-E1 myp2 $\Delta$* , *myo2-E1-Sup1 myp2 $\Delta$* , and *myp2 $\Delta$*  strains to investigate aspects of actomyosin ring function. The time taken for ring assembly and contraction and the ring contraction rate were comparable in wild-type and *myp2 $\Delta$*  cells (Figures 2B–2F), clarifying that Myp2p is not important for either ring assembly or contraction at  $36^\circ\text{C}$  when Myo2p is fully functional. *myo2-E1 myp2 $\Delta$*  assembled abnormal actomyosin rings that underwent abnormal disassembly (Figures 2B and 2C). *myo2-E1-Sup1 myp2 $\Delta$*  assembled actomyosin rings of normal appearance, and the assembly of these rings took  $\sim 6$  min more than wild-type and *myp2 $\Delta$*  cells (Figures 2B–2D). Ring contraction was dramatically affected in *myo2-E1-Sup1 myp2 $\Delta$*  (Figures 2B, 2C, 2E, and 2F). Contraction and disassembly took more than twice the amount of time compared to wild-type cells, while the ring contraction rate was less than half of that observed in wild-type cells (Figures 2E and 2F). Furthermore, contraction was frequently asymmetric and led to rings disassembling abnormally and often to the fragmentation of the ring into two or more clusters (Figures 2B, time points 48–72 min, and 2C). Since *myo2-E1-Sup1 myp2 $\Delta$*  and *myo2-E1-Sup1* were capable of actomyosin ring assembly but showed appreciable defects in ring contraction, we conclude Myo2p activity is essential for ring assembly and contraction, whereas Myp2p plays an ancillary role in promoting inefficient contraction when Myo2p motor activity is compromised at  $36^\circ\text{C}$  (compare ring contraction times and rates between *myo2-E1-Sup1* and *myo2-E1-Sup1 myp2 $\Delta$*  in Figures 2E and 2F).

Analysis of three-dimensional structures of rigor myosin (actin bound: 4A7F) and blebbistatin-bound myosin (actin unbound: 1YV3) suggested that the amino acid substitutions in *myo2-E1-Sup1* may result in increased binding affinity toward F-actin (Figure S2; see the Supplemental Experimental Procedures for a detailed description of the structural analysis). This in turn may lead to defective actomyosin ring contraction due to *myo2-E1-Sup1* being tightly bound to actin, leading to an actin filament translocation defect.

We have already developed methods to isolate ATP-dependent contraction-competent actomyosin rings [8, 18]. We therefore used this system to test if isolated actomyosin rings in cell ghosts from *myo2-E1-Sup1* were capable of ATP-dependent contraction. Actomyosin rings were isolated from wild-type, *myo2-E1*, *myp2 $\Delta$* , *myo2-E1 myp2 $\Delta$* , *myo2-E1-Sup1*, and *myo2-E1-Sup1 myp2 $\Delta$*  cells grown at the permissive temperature of  $24^\circ\text{C}$ . Actomyosin rings isolated from wild-type and *myp2 $\Delta$*  cells underwent normal and rapid contraction upon ATP addition (Figures 3A and 3B). As previously reported [8], upon the addition of 0.5 mM ATP, actomyosin rings isolated from *myo2-E1* and *myo2-E1 myp2 $\Delta$*  either contracted slowly or underwent fragmentation (Figures 3A and 3B). Interestingly, despite the moderate delay in ring assembly timing, actomyosin rings of normal appearance assembled in *myo2-E1-Sup1* and *myo2-E1-Sup1 myp2 $\Delta$*  at the restrictive temperature. However, rings isolated from these strains did not contract normally, even at the permissive temperature for *myo2-E1* ( $24^\circ\text{C}$ ). Instead, rings from these strains remained stable and broke into large fragments. These experiments established that, consistent with *in vivo* results, rings isolated from *myo2-E1-Sup1* and *myo2-E1-Sup1 myp2 $\Delta$*  cells are defective in ATP-dependent contraction *in vitro*. These results were consistent with the idea that the product of *myo2-E1-Sup1* is defective in its motor activity and actin filament translocation, but not in actin filament binding, which in turn may explain the ability of *myo2-E1-Sup1* to support actomyosin ring assembly, but not contraction. However, it was possible that the actin translocation defect in *myo2-E1-Sup1* was due to allosteric effects on other unidentified components of the actomyosin ring that affect ring contraction, rather than a direct effect of *myo2-E1-Sup1* on actin filament translocation.

To distinguish between these possibilities, we purified the products of *myo2<sup>+</sup>*, *myo2-E1*, and *myo2-E1-Sup1* using an expression system developed by Lord and Pollard [11]. *Myo2-E1-Sup1p* was more difficult to purify (potentially due to its tight binding to actin) and was eventually isolated from Latrunculin A-treated cells (Figure S3A). We then performed actin motility assays as described in Lord and Pollard [11]. In brief, Myo2p and the mutant versions were immobilized on nitrocellulose-coated coverslips, overlaid with rhodamine-phalloidin-stabilized rabbit actin filaments, and incubated with ATP (Figures 4A, 4B, S3B, and S3C; Movies S1, S2, S3, and S4). We found that wild-type Myo2p was able to bind and translocate actin filaments at  $\sim 0.72 \pm 0.13$   $\mu\text{m}/\text{s}$  when incubated with ATP. Also, as previously reported [11], *Myo2-E1p* did not attach to actin filaments (Movie S2). Interestingly, unlike the product of *myo2-E1*, the product of *myo2-E1-Sup1* bound actin tightly, since these filaments were either severely affected for motility or were non-motile (gliding velocity was



**Figure 2. *myo2-E1-Sup1* Fails in Actomyosin Ring Contraction in the Absence of the Non-essential Myosin Heavy Chain *Myp2p***

(A) Serial dilutions (10-fold) of wild-type, *myo2-E1*, *myp2Δ*, *myo2-E1 myp2Δ*, and *myo2-E1-Sup1 myp2Δ* were spotted onto YEA plates and grown for 3 days at 24°C and 36°C.

(B) Time-lapse series of wild-type, *myp2Δ*, *myo2-E1 myp2Δ*, and *myo2-E1-Sup1 myp2Δ* cells expressing 3GFP-tagged myosin regulatory light chain (Rlc1-3GFP) as a contractile ring marker and mCherry-tagged tubulin (atb2-mCherry) as a cell-cycle stage marker. Cells were grown at 24°C and shifted to 36°C ( $t = 0$  indicates the time before Rlc1-3GFP nodes localize to the cell middle). Images shown are maximum-intensity projections of z stacks. Scale bars represent 3 μm.

(C) Kymographs of a 3D-projected ring from wild-type, *myo2-E1 myp2Δ*, and *myo2-E1-Sup1 myp2Δ* cells. Scale bars represent 3 μm.

(D) Timing of actomyosin ring assembly from nodes. Quantification of (B) is shown. Asterisks indicate the statistical significance of the difference between the different genotypes compared to the wild-type. Statistical significance was calculated by Student's t test (\*\*\*\* $p < 0.0001$ ). Error bars represent SD.

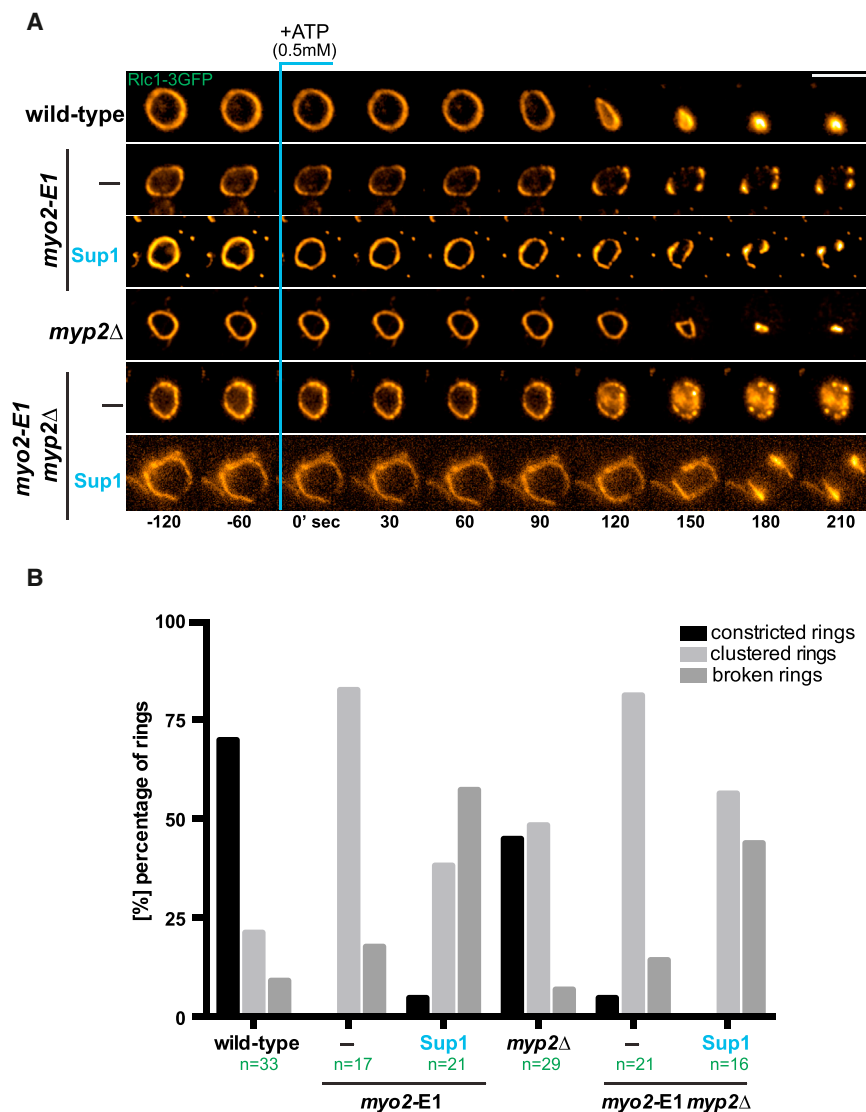
(E) Timing of actomyosin ring contraction. Quantification of Figure 1C and (B) is shown. Statistical significance was calculated by Student's t test (\*\*\*\* $p < 0.0001$ ). Error bars represent SD.

(F) Constriction rate determined from a graph of ring circumference versus time. Contraction rates of Figure 1C and (B) are shown. Statistical significance was calculated by Student's t test (\*\*\*\* $p < 0.0001$ ). Error bars represent SD.

See also Figure S2.

$\sim 0.06 \pm 0.04$  μm/s). *Myo2-E1-Sup1p* also had a dominant effect when mixed with wild-type *Myo2p*. The mixture bound to actin filaments but these filaments were non-motile. The fact that *Myo2-E1-Sup1p* did not support motility, despite binding actin filaments and its dominant-negative effect on motility over wild-type *Myo2p*, suggests that *Myo2-E1-Sup1p* is most likely a novel rigor mutant of *Myo2p*.

Our work reported in this study establishes that the type II myosin, *Myo2p*, plays two distinct and essential roles. Since cells harboring the novel rigor mutant allele *myo2-E1-Sup1* assemble normal actomyosin rings, despite the defective contraction in vitro and in vivo, it is possible that actomyosin ring assembly depends on the ability of *Myo2p* to cross-link actin filaments. Actomyosin ring assembly in *myo2-E1-Sup1* cells is



### Figure 3. Isolated Actomyosin Rings of *myo2-E1-Sup1* Do Not Undergo ATP-Dependent Contraction

(A) Cell ghosts were prepared from wild-type, *myo2-E1*, *myp2Δ*, *myo2-E1 myp2Δ*, *myo2-E1-Sup1*, and *myo2-E1-Sup1 myp2Δ* grown at 24°C. Ring contraction experiments were performed at 24°C and contraction was activated by the addition of 0.5 mM ATP. Images shown are maximum-intensity projections of z stacks. Scale bars represent 5 μm.

(B) Graph showing percentage of contracted, clustered, and broken rings. Quantification of (A) is shown.

See also Figure S2.

slower than in wild-type cells (possibly due to cross-linking and tighter binding of Myo2-E1-Sup1p with actin), suggesting that myosin II motor activity may also play a role in actomyosin ring assembly, as previously proposed [19, 20]. It is possible that clustering of cytokinetic precursor nodes can occur through tension generated by myosin II-dependent cross-linking of actin filaments. This view is consistent with aspects of the work of Ma and colleagues who have proposed that actin translocation activity of myosin II is not essential for cytokinesis [4]. Inconsistent with the work of Ma and colleagues, however, are our findings that actomyosin rings in *myo2-E1-Sup1* cells do not contract normally, that actomyosin rings isolated from those cells fail to undergo ATP-dependent contraction, and that one-step-purified Myo2-E1-Sup1p does not support ATP-dependent actin filament motility in vitro. These observations suggest that myosin II motor activity is essential for actomyosin ring contraction.

Thus, through the analysis of novel myosin II mutant alleles, we have been able to discriminate between myosin II motor activity-dependent and -independent steps in cytokinesis. Published

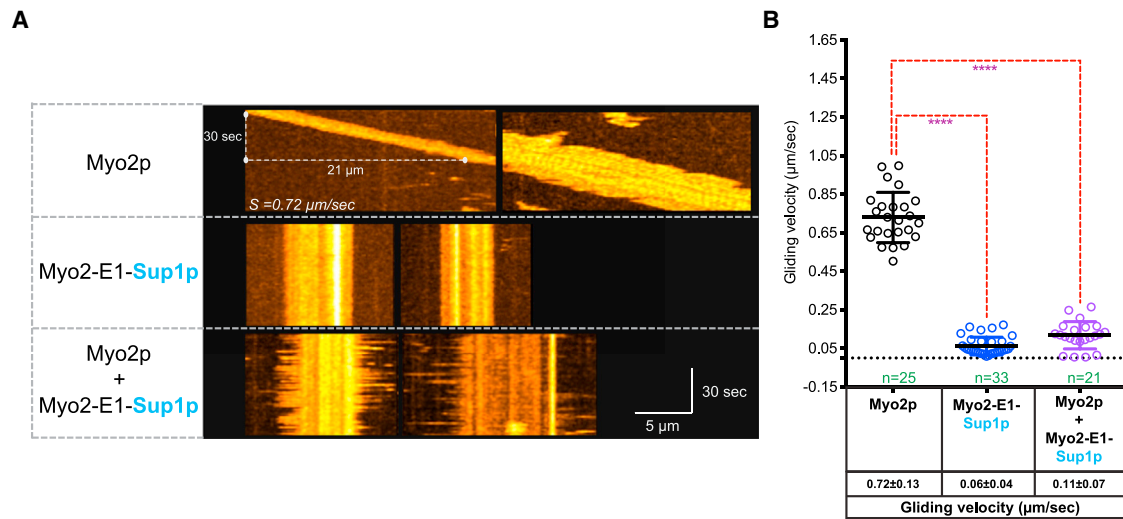
work in *S. cerevisiae* and mammalian cells [4, 5, 21] has questioned the role of myosin II motor activity in cytokinesis. It is likely that in some cell types, tension generated by actin filament cross-linking and filament disassembly alone may suffice for cytokinesis, whereas in others such as *S. pombe*, cytokinesis may depend on motor activity-dependent and -independent functions of myosin II.

#### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and four movies and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2017.01.028>.

#### AUTHOR CONTRIBUTIONS

S.P. conceived and designed experiments, acquired data, performed analysis and interpretation of data, and drafted/revised the article. S.R. and M.M. generated yeast strains and performed preliminary analysis. T.G.C., A.K., S.H., B.C.L., M.S., and R.H. performed analysis and interpretation of data



**Figure 4. Myo2-E1-Sup1p Showed Tighter Actin Binding but No Motility**

(A) Type II Myosin-based actin filament-gliding assay. Representative kymographs of time-lapse fluorescence micrographs of actin filaments labeled with rhodamine-phalloidin are shown. Scale bars represent 5  $\mu\text{m}$ .

(B) Quantification of the actin filament-gliding assay of (A). Different myosins (Myo2p, Myo2-E1-Sup1p, and Myo2p + Myo2-E1-Sup1p) were tested for gliding velocity ( $\mu\text{m/s}$ ) using rhodamine-phalloidin-labeled actin.

See also Figure S3 and Movies S1, S2, S3, and S4.

and generated yeast strains and reagents. P.G. performed structural analysis and interpretation of data and drafted/revised the article. M.K.B. conceived the project, conceived and designed experiments, and performed analysis and interpretation of data. S.P. and M.K.B. wrote the manuscript. All authors reviewed the manuscript.

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