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Tracking the Importance of Tropomyosin in Myosin-II- and Myosin-V-Dependent Processes in Fission Yeast

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Actin filaments function in an ever-expanding list of cellular processes including cell motility, intracellular transport, cytokinesis, and endocytosis. A variety of specialized actin filament structures are established within the same cell to accomplish different tasks. Such actin structures must operate independently of one another, dictating the need for precise regulation to ensure appropriate functional outputs. These different actin structures specify the involvement of certain actin-binding proteins, such as actin-based myosin motors [1].

From a technical standpoint, the tractability and relative simplicity of the fission yeast (*Schizosaccharomyces pombe*) system makes it an appealing model with which to study myosin specification. Fission yeast utilizes three distinct actin structures during vegetative growth and possesses five myosins [2]: a class I myosin (Myo1p), two class IIs (Myo2p and Myp2p), and two class Vs (Myo51p and Myo52p). Actin 'patches' rely on Myo1p and an Arp2/3-mediated, branched actin filament network that helps drive internalization of endocytic vesicles. Actomyosin contractile rings drive cytokinesis and are made up of for min-mediated, unbranched actin filaments arranged in short bundles and employ myosin-II (Myo2p and Myp2p) and Myo51p. Actin 'cables' are made up of long, formin-mediated, unbranched actin bundles that provide tracks for Myo52p-mediated transport.

Mechanisms must be in place to insulate these different actin structures from one another. With respect to actomyosin function, why do myosin-II and myosin-V motors target unbranched, formin-mediated actin filaments, as opposed to the Arp2/3-mediated, branched filament networks? Fission yeast tropomyosin (Cdc8p) is likely a key determinant of actin function given its preferential accumulation at actin rings and cables [3]. We used fluorescence microscopy-based approaches to test the importance of tropomyosin in myosin-II (Myo2p) and myosin-V (Myo52p) dynamics in live fission yeast cells.

Myo2p contractile ring assembly and constriction was tracked by epi-fluorescence microscopy using a Nikon TE2000-E2 inverted microscope with motorized fluorescence filter turret and a Plan Apo 60x (1.45 NA) objective. Fluorescence utilized an EXFO X-CITE 120 illuminator; images were captured with a Photometrics CoolSNAP HQ2 14-bit camera. Myo2p (Rlc1p-GFP) ring dynamics were imaged relative to mitotic progression using an SPB (spindle pole body) marker (Sad1p-GFP) to track spindle elongation and breakdown. Analysis was performed using Image J and Microsoft Excel.

Tracking of Myo52p-3xYFP particle motility along actin cables utilized a Nikon Eclipse Ti-U inverted microscope equipped with a 100x PlanApo objective lens (1.40 NA) for through-the-objective near-TIRF (total internal reflection fluorescence) microscopy. The YFP was excited with a 473 nm laser line. The angle at which the laser entered the objective was tuned such that the illumination was not perfect TIRF, allowing the laser to excite the entire thickness of the cell, yet resulting in a better signal to noise ratio than epi illumination. Images were obtained using a Stanford Photonics XR/Turbo-Z camera. Movement of YFP particles over time was tracked manually using ImageJ.

We assessed the impact of tropomyosin Cdc8p on myosin dynamics and function using a temperature-sensitive *cdc8* mutant (*cdc8-27*). Rlc1p-GFP (myosin-II) and Myo52p-3xYFP (myosin-V) were tracked in wild-type and mutant cells (grown and analyzed at permissive growth temperatures, 25-30°C). Our data indicated that Cdc8p function was important for the timely assembly of actomyosin rings, while having no effect on the initiation and rate of ring constriction (Figure 1). Perturbation of Cdc8p function slowed down the speed and reduced the run length of Myo52p particles (Figure 2). The frequency of Myo52p particle transport events were also significantly reduced (Figure 2). Collectively, our data suggests that the presence of tropomyosin on unbranched actin filaments helps sort and direct myosin-II and myosin-V motor function. *In vitro* studies with purified proteins are being employed to test this hypothesis.

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[4] The authors acknowledge funding from the NIH (GM097193) and the AHA (0835236N).

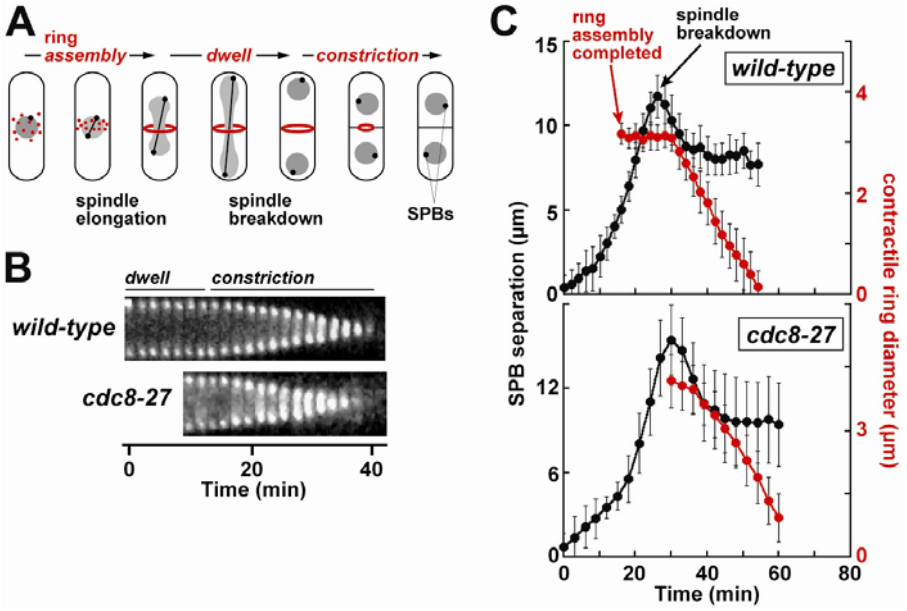

Figure 1. A) Schematic illustrating the timing of wild-type actomyosin ring assembly and constriction relative to mitotic progression (as charted by SPB separation). B) Representative kymographs from wild-type and *cdc8-27* cells tracking ring (Rlc1p-GFP) lifetimes and diameters. C) Plots summarizing the dynamics (dwell and constriction phases) of assembled wild-type and *cdc8-27* rings relative to mitotic progression (n=30-50). Note the delay in assembly in *cdc8-27* cells.

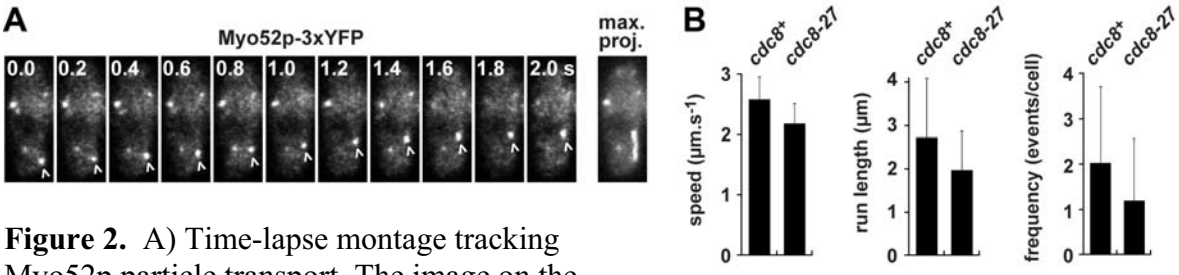

Figure 2. A) Time-lapse montage tracking Myo52p particle transport. The image on the Right is a maximum projection of the montage showing the particle trajectory. B) Histograms showing the altered speed, run length, and (most strikingly) frequency of Myo52p transport in *cdc8-27* cells.

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