Supplemental Materials
Molecular Biology of the Cell

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SUPPLEMENTAL MATERIALS

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Determination of myosin-II load-free velocity $v_{\text{myo}}^0$ from the gliding filament assay of (Stark et al., 2010)

A previous experiment measured the velocity of actin filaments gliding on fission yeast myosin-II Myo2 that was adhered onto a coverslip (Stark et al., 2010). The authors measured the increase of the gliding velocity of the filaments with the number of Myo2 heads interacting with the actin filament. The velocity saturated at ~50 heads.

Using the measurement of a mean of 1500 and 180 dimers of myosin Myo2 and formin Cdc12 respectively using quantitative fluorescence microscopy (Wu and Pollard, 2005; Courtemanche et al., 2016), and the proposal of a mean of 8 myosin Myo2 dimers per node using FPALM (Laplante et al., 2016), we calculated the total mean number of nodes as 190, and a mean of 0.95 formin dimers per node. Thus, our model has 190 nodes with one actin filament, one formin dimer, and 8 myosin dimers each. Thus, the ratio of Myo2 molecule number to actin filament number is 16. At this ratio, the gliding filament assay measurements report an actin filament gliding velocity of 240 nm s$^{-1}$, which is the myosin-II load-free velocity $v_{\text{myo}}^0$ as the myosins experience little load in these experiments (Stark et al., 2010).

Linear stability analysis of the model-predicted homogeneous ring in the presence of turnover

One steady state solution to Eqns. 1-4, M.1-M.4 is $\rho_\pm (x,t) = \rho_0 / 2$ and $v_\pm (x,t) = \pm v_0$ where $v_0 = \rho_0 f_{\text{node}} l / \gamma_{\text{anc}}$. Now, let us consider a small perturbation to the steady state of wavenumber $k$ or equivalently a wavelength $2\pi / k$. The node density is $\rho_\pm (x,t) = \rho_0 / 2 + \varepsilon_\pm (t) \exp(i k x)$ where $\varepsilon_\pm (0) \ll \rho_0$ and we calculate the time evolution of $\varepsilon_\pm (t)$. Plugging this into Eqns. 1-4, M.1-M.4 and linearizing about the steady state $\rho_\pm (x,t) = \rho_0 / 2$, we get

$$\frac{\partial \varepsilon_+}{\partial t} = -a_1 \varepsilon_+ - a_2 \varepsilon_- \quad \text{(S1)}$$
$$\frac{\partial \varepsilon_-}{\partial t} = -a_2^* \varepsilon_+ - a_1^* \varepsilon_- \quad \text{(S2)}$$

where $a_1 = ikv_0 - (1 - \cos kl) / 2\tau_a + 1 / \tau_{\text{turn}}$, $a_2 = (\exp ikl - 1) / 2\tau_a$, and $\tau_a = l / 2 v_0$ is a characteristic timescale whose meaning will be explained later in the section. Eliminating $\varepsilon_-$ between these two equations, we get

$$\frac{\partial^2 \varepsilon_+}{\partial t^2} + (a_1 + a_1^*) \frac{\partial \varepsilon_+}{\partial t} + (|a_1|^2 - |a_2|^2) \varepsilon_+ = 0 \quad \text{(S3)}$$

The solution to this equation is of the form $\varepsilon_+ (t) = c_1 \exp -t / \tau_1 + c_2 \exp -t / \tau_2$ where $\tau_1^{-1}$ and $\tau_2^{-1}$ are the solutions of the quadratic equation $x^2 + (a_1 + a_1^*) x + (|a_1|^2 - |a_2|^2) = 0$ as can be
verified by plugging in this solution to Eq. S3. The constants \(c_1\) and \(c_2\) depend on the initial conditions \(\varepsilon_\pm(0)\) and are not calculated here.

The timescales \(\tau_1, \tau_2\) set the stability of the ring. Using the substitution \(\tau_a = l/(2v_0)\) and the expressions for \(a_1, a_2\), the solutions to the quadratic equation are

\[
\frac{1}{\tau_1} = \frac{1}{\tau_{\text{turn}}} - \frac{1-\cos kl}{2\tau_a} + ikf(k) \tag{S4}
\]

\[
\frac{1}{\tau_2} = \frac{1}{\tau_{\text{turn}}} - \frac{1-\cos kl}{2\tau_a} - ikf(k) \tag{S5}
\]

where \(f(k) = v_0((k^2l^2/2 - 1 + \cos kl)/(k^2l^2/2))^{1/2}\). Thus, the fluctuations could decay or grow exponentially with time depending on whether the real part of the timescales \(\text{Re}(\tau_1), \text{Re}(\tau_2)\) is positive or negative respectively. From Eqns. S4 and S5, we can see that if \(\tau_{\text{turn}} < \tau_a\), the real part is positive for all wavenumbers and the model-predicted homogeneous ring is stable in the face of small fluctuations. As the experimentally measured turnover time \(\tau_{\text{turn}} = 18.6\, s\) is smaller than the model-predicted aggregation time \(l/2v_0 = 61.4\, s\), this condition is satisfied. In addition, as the turnover time is only about a third of the aggregation time i.e. \(\tau_{\text{turn}} \ll \tau_a\), fluctuations of all wavelengths decay with roughly the same time scale i.e. \(\text{Re}(\tau_1) \approx \text{Re}(\tau_2) \approx \tau_{\text{turn}}\).

In the absence of turnover, the real parts of these time scales are negative and the fluctuations grow with time. The shortest time scale of growth is for a fluctuation of wavelength \(2l\) and is \(\tau_a\), as can be seen by the substitution \(k = 2\pi/(2l)\) in the solutions above. The fastest growing fluctuations are those of wavelengths \(2l/n\) where \(n\) is an odd integer. We note here that this analysis is only valid for the initial stages of growth in fluctuation amplitude where these amplitudes are small compared with the mean node density \(\rho_0\). In the later phase of growth, non-linear effects are important.

**Effect of Myo2 force-velocity relation and actin filament growth on myosin force per head**

For simplicity our model considered a fixed force per Myo2 head, \(f_{\text{myo}}\), which was assumed to have the same order of magnitude as the stall force of Myo2, \(f_{\text{stall}}\). However, we did not consider the myosin force-velocity relationship to estimate how much \(f_{\text{myo}}\) deviates from \(f_{\text{stall}}\). Our model did not explicitly include formin-mediated polymerization of actin filaments, which would increase this deviation. Below, we calculate by how much \(f_{\text{myo}}\) differs from \(f_{\text{stall}}\).

(i) Consider first interfamily interactions, i.e. between nodes whose actin filament has one polarity with nodes whose filament has the opposite polarity. In our model, the relative velocity between myosins of one node family and actin filaments belonging to nodes of the opposite polarity is \(2v_0\), where \(v_0\) is the node velocity. Actin filament growth at rate \(v_{\text{pol}}\) would increase this relative velocity to \(2v_0 + v_{\text{pol}}\). Assuming a simple linear force-velocity relation, the myosin force is thus lowered to \(f_{\text{myo}} = f_{\text{stall}}(1 - [2v_0 + v_{\text{pol}}]/v_{\text{myo}}^0)\). (ii) Now consider intrafamily
interactions, between two nodes of the same polarity. The myosin force is now lowered to
\[ f_{\text{myo}} = f_{\text{stall}} \left( 1 - \frac{v_{\text{pol}}}{v_{\text{myo}}^0} \right), \]
as the relative velocity between myosins belonging to the same node family is zero, so the relative velocity between the myosin of one node and the actin filament of another node in the same family is \( v_{\text{pol}} \). (iii) To obtain the overall effect on the value of \( f_{\text{myo}} \), it is necessary to take the mean of the two contributions from interfamily and intrafamily interactions, since they contribute equally to ring tension. This gives a mean relation
\[ f_{\text{myo}} = f_{\text{stall}} \left( 1 - \frac{v_0 + v_{\text{pol}}}{v_{\text{myo}}^0} \right). \]

Now we estimate filament growth rates using previous experimental measurements of ring disassembly in the presence of the actin monomer sequestering drug Latrunculin A. Only \( \sim 10\% \) of rings remained after 55 s of exposure (Yonetani et al., 2008). This gives an actin turnover rate of \( \sim 0.042 \text{ s}^{-1} \), assuming the fall-off of actin subunit numbers in the ring is exponential with time. Using this turnover rate, the actin filament growth rate that would have normally occurred to synthesize a filament of mean length 2.7 \( \mu \text{m} \) is \( v_{\text{pol}} \sim 110 \text{ nm s}^{-1} \).

Using this value of \( v_{\text{pol}} \), the node speed \( v_0 = 22 \text{ nm s}^{-1} \), and the Myo2 load-free velocity \( v_{\text{myo}}^0 = 240 \text{ nm s}^{-1} \), we obtain \( f_{\text{myo}} \sim 0.45f_{\text{stall}} \). This is our main conclusion regarding how the force-velocity relation and actin polymerization reduce the myosin per force per head from the stall force value.

Now in the main text (subsection “Tension is generated in the cytokinetic ring by myosin pulling on barbed end anchored actin filaments” of Results) we compared the model-predicted value of ring tension with the experimentally measured value, and we found \( f_{\text{myo}} \sim 1.1 \text{ pN} \). Thus, we obtain a value of the stall force \( f_{\text{stall}} \sim 2.4 \text{ pN} \). This is close to previously reported stall force values for myosin-II in different organisms, 0.6 – 2.3 pN (Kishino and Yanagida, 1988; Molloy et al., 1995; Ishijima et al., 1996; Tyska et al., 1999).
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