SUPPLEMENTAL MATERIALS

SUPPLEMENTAL MATERIALS AND METHODS

Computational model

Summary

In order to study the effect of crosslinkers on filament capture by nodes, we constructed a computational model, based on the Search-Capture-Pull mechanism described by Vavylonis and coworkers (Vavylonis et al., 2008). To produce our model, we started from the previously described Monte Carlo simulation methodology (Vavylonis et al., 2008). We then introduced segmented filaments and crosslinkers within this Monte Carlo framework, very similar to a previously described model (Laporte et al., 2012). The principal differences between our work and Laporte et al. (Laporte et al., 2012) are that those authors used Langevin-dynamics as opposed to a Monte Carlo simulation, and our rules for crosslinking and the implementation of motors are slightly different (described below). However, the motor model does not affect the results of this work, as we focused on filament capture efficiency (Figure 8D), and included motor dynamics in our model only for visualization of node condensation shown in Supplemental Video 9. We emphasize that the purpose of the model in this paper is not to produce quantitatively accurate predictions, but to demonstrate that a physically reasonable proxy model for the experiments supports the hypothesis of dynamic crosslinkers increasing the efficiency of the Search-Capture-Pull process.

Filaments extend from two formin-associated beads (nodes) positioned 600 nm apart, which is approximately the mean distance in fission yeast (Laporte et al., 2011). Filaments are segmented lines composed of crosslinker binding sites spaced 35 nm apart, the approximate distance between available binding sites in an α-actinin bundle (Meyer and Aebi, 1990). In our model, crosslinking can occur between two different filaments when two crosslinker binding sites are separated by approximately 20 nm (see below). Filaments extend at a rate 175 nm/sec, the approximate rate in vivo (Vavylonis et al., 2008), such that new segments can be produced on integer time steps. For each step of the simulation time is advanced by \( dt = 0.05 \) sec. Filaments are initially nucleated at an angle \( \theta \) above the horizon, and the angular dependence of filament crosslinking on the Search-Capture efficiency is investigated. Simulations were run up to a maximum time of \( \approx 10 \) sec (200 simulation steps) such that filaments extend to three times the initial distance between the beads. If capture has not occurred by that point, the capture process is considered to be a failure.

We computed the “rate of first filament capture”, \( k_{\text{first-hit}} \), by averaging \( \frac{1}{t_{\text{first-hit}}} \), where \( t_{\text{first-hit}} \) is either the first time at which one of the actin filaments hits the other “node” or is considered infinite if the simulation ends without capture. We studied the system in two spatial dimensions as reported previously (Vavylonis et al., 2008). We expect the increase in efficiency of node capture due to crosslinking would be even larger in three dimensions, as it is an intrinsically harder search process with only simple filament diffusion.
Filament model
Actin filaments are treated as bead-spring polymers of segment length $l_s$. The energy of a filament is given by,

$$E_{\text{filament}} = E_{\text{bend}} + E_{\text{stretch}} = \sum_{i=1}^{N_{\text{segments}}-1} \frac{1}{2} k_{\text{bend}} \theta_i^2 + \sum_{i=1}^{N_{\text{segments}}} \frac{1}{2} k_{\text{stretch}} (l_i - l_s)^2,$$

where $l_i$ is the current length of segment $i$, and $\theta_i$ is the angle between segments $i$ and $i+1$.

$k_{\text{bend}}$ is given by the equation $k_{\text{bend}} = k_B T L_p/(2l_s)$, where $k_B$ is Bolzmann’s constant and $L_p$ is the persistence length of actin, which we take to be 10 $\mu$m (Gordon et al., 2012). $k_{\text{stretch}}$ is derived from the Young’s modulus of actin, which is approximately $E = 2 \times 10^9 N/m^2 = 2000 pN/nm^2$, with a cross-sectional area of $S = 25 nm^2$. $k_{\text{stretch}} = E S / l_s = 50,000 pN/l_s$. We find for computational efficiency (which is in general limited by the stiffest spring in the system) we must decrease this spring constant, as reported previously (Laporte et al., 2012). In our final data, we used $k_{\text{stretch}} = 680 pN/l_s$, but we found that the simulation results do not depend much on this (note, a previous report used a value of $k$ that is 200 times smaller than the value estimated from Young’s modulus (Laporte et al., 2012)).

At every time step where filament positions are adjusted, we made $N_{\text{moves}}$ attempts to move each of the $N_{\text{beads}}$ beads in each filament by a Monte Carlo procedure. We chose a bead at random, and chose a random direction to move in the range $[0,d_{\text{max}}]$, for both $x$ and $y$, where we chose $d_{\text{max}}=0.04 l_s$. Each move was accepted or rejected based on a Metropolis criterion (Frenkel and Smit, 2002). This $d_{\text{max}}$ results in an average acceptance ratio of approximately 35%. To allow for collective motion of two filaments when bound, we alternate times between the two filaments making $N_{\text{moves}} N_{\text{beads}} / 5$ attempts at each stage of this sampling.

In these data we used $N_{\text{moves}}=100$, which appears sufficient to allow filaments to relax and have fluctuations similar to what we observe in experiments. We have also repeated some of these tests with other values of $N_{\text{moves}}$ with no significant change to the results.

Crosslinker model
Crosslinkers are additional harmonic interactions between beads on actin filaments. Crosslinkers have length $l_x = 20 nm$ in accordance with the SpAin1 crosslinker studied in this work. We varied the crosslinker attachment lifetime $\tau_x$ and on rate $k_{\text{on}}$. We denote a dimensionless binding constant, $K_d^{-1} = k_{\text{on}} \tau_x$. The probability of crosslinking two filaments must depend on the distance between two beads, as does the probability of detachment. We chose a Gaussian distribution of probability for attaching and Bell’s law for detachment (Nedelec and Foethke, 2007), where we assume the crosslinker is also a harmonic spring with stretch force $f_x$. In that case,

$$f_x(l) = -k_x (l - l_s)$$

$$P_{\text{off}}(l) = \frac{dt}{\tau_x} e^{\frac{|f_x(l)|}{k_x}}$$

$$P_{\text{on}}(r_{\text{bead}}) = k_{\text{on}} dr_{\text{bead}} \left(\frac{r_{\text{bead}} - l_s}{P_{\text{on}}(r_{\text{bead}})}\right)^2$$
We chose $k_x = 10 \text{ pN/nm}$ (A previous report used 500 pN/nm (Laporte et al., 2012)), $F_x = 10 \text{ pN}$ and $r_x^{\text{cut}} = 5 \text{ nm}$, which produce reasonable crosslinking dynamics.

Each crosslinker adds an energy

$$E_x = \frac{1}{2} k_x (l - l_x)^2$$

to the total energy of the system, which is included in the metropolis criterion for filament dynamics.

**Filament extension**

Segments are added at the node end of filaments every $l_s/(v_{pol}dt)$ steps. Before any crosslinking or capture has occurred, a segment is added by translating the entire filament by $l_s$ along the direction that the first segment is pointing out of the bead. Once the filament is bound somewhere along its length, the filament is extended in $N=5$ stages, where the second bead in the filament is translated $l_s/N$ along the vector of the first segment, and beads 2-$N$ are sampled $N_{\text{moves}}$ times to relax the filament. After these N stages, a new bead is inserted at the original position of the second bead.

The force on the node is considered the stretching energy between filament beads one and two. Filament extension stops if this force exceeds a cutoff $F_{\text{stall}}$, as reported previously (Vavylonis et al., 2008), which in these results was set to 10 pN. However, this does not affect the results presented in the paper, as this occurs after filament capture.

**Capture, myosin dynamics, and node movement**

The minimum distance between an actin filament and the other formin node is computed. If this distance is less then $r_{\text{capture}}=100 \text{ nm}$, then the “myosin” position is assigned to the closest bead in the actin filament, and a spring of zero-rest-length attaches the filament to its relative initial capture position. A new energy is added to the total energy of the system (with a corresponding force $F_{\text{myo}}$).

$$E_{\text{myo}}(l_{\text{myo}}) = \frac{1}{2} k_{\text{myo}} l_{\text{myo}}^2$$

Once myosin is attached, it moves $v_{\text{walk}}dt$ along the filament at each time step, where $v_{\text{walk}}$ is given by the formula

$$v_{\text{walk}} = v_{\text{myo}} \left( 1 + \left( \frac{F_{\text{myo}}}{F_{\text{myo}}^{\text{max}}} \right) \right)$$

following [Gordon] where $v_{\text{myo}}$ is the bare myosin walking speed, which we set to 400 nm/sec (Vavylonis et al., 2008). We used $k_{\text{myo}} = 2 \text{ pN/nm}$ and $F_{\text{myo}}^{\text{max}} = 20 \text{ pN}$. In general, these parameters should be tuned to match experimental results, but for this work, because the myosin dynamics occur only after the capture process, we chose parameters that were qualitatively reasonable.

After the myosin is moved along the filament, stretching the myosin’s internal spring. The filament is sampled $N_{\text{moves}}$ times. This allows the myosin to relax and pulls the filament through the myosin attachment position.

The action of the myosin in the previous paragraph stretches the actin filament, which can produce a force on the other node (the stretching force between the first two beads at the formin end of the filament) as
described in the previous section. This force $F_{\text{bead}}$ is translated into a velocity with viscosity $\eta = 0.2 \text{ pN sec/nm}$ as previously described (Vavylonis et al., 2008), $v_{\text{bead}} = F_{\text{bead}}/\eta$, which results from assuming dynamics of the beads are over-damped.

In these simulations, the bead is moved by the vector $d = v_{\text{bead}} dt$ in 15 stages where the first bead in the attached actin filaments is moved along with the node, and then attached filaments are sampled $N_{\text{moves}}$ times to relax their positions.

For the experiments described in Figure 8, C and D and Supplemental Video 9, the nodes were taken to have diameter 150 nm, and the simulation terminates when nodes touch.
### Table S1. *S. pombe* Strains Used in this Study

| Strain  | Genotype                                                                 | Source/Reference          |
|---------|---------------------------------------------------------------------------|---------------------------|
| FY527   | h-, leu1-32, his3-D1, ura4-D18, ade6-M216                                  | S. Forsburg\(^a\)         |
| FY528   | h+, leu1-32, his3-D1, ura4-D18, ade6-M210                                  | S. Forsburg\(^a\)         |
| KV628   | h-, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; ade6-M210, leu1-32, ura4-D18  | This study                |
| KV717   | h-, ain1-GFP-KanMX6, leu1-32, ura4-D18                                    | This study                |
| KV734   | h-, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; ade6-, leu1-32, ura4-D18,     | This study                |
|         | Pmnt41-SpAin1-mGFP::Leu+                                                  |                           |
| KV736   | h-, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; ade6-, leu1-32, ura4-D18,     | This study                |
|         | Pmnt41-SpAin1(R216E)-mGFP::Leu+                                           |                           |
| KV761   | h?, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; leu1-32, ura4-D18, ade6-,     | This study                |
|         | Pmnt81-SpAin1-mGFP::Leu+                                                  |                           |
| KV762   | h?, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; leu1-32, ura4-D18, ade6-,     | This study                |
|         | Pmnt81-SpAin1(R216E)::Leu+                                                |                           |
| KV769   | h-, rlc1-tdTomato-natMX6, ain1-GFP-KanMX6, leu1-32                        | This study                |
| KV960   | h?, myo2-E1, ain1-Δ1:: kanMX6, rlc1-tdTomato-natMX6; ura4-D18, ade6-M21?, | This study                |
|         | Pmnt81-SpAin1(R216E)-mGFP::Leu+                                           |                           |
| JW1341  | h-, rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18                       | (Laporte et al., 2012)    |
| JW1564  | h+, ain1-Δ1:: kanMX6, ade6-M210, leu1-32, ura4-D18                         | (Laporte et al., 2012)    |

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**Supplemental Figure S1.** Alignment of representative α-actinin isoforms. (A) Domain organization of SpAin1 and HsACTN4. Dashed lines indicate significant residue identity between specific spectrin repeats (SR). ABD: Actin Binding Domain. (B) Alignment of ABDs. Sp: Schizosaccharomyces pombe (CAB10105.1); Dm: Drosophila melanogaster (AAN09068.1); Gg: Gallus gallus (AAA48570.1); Hs: Homo sapiens α-actinin2 (HsACTN2; AAA51583.1) and α-actinin4 (HsACTN4; A4K467). Blue, CH1. Orange, CH2. Red and asterisk below, the mutated amino acid in SpAin1(R216E). Residues with ≥90% amino acid similarity are highlighted in grey. (C and D) Alignment of spectrin repeat (SR) regions. (C) Alignment of SpAin1(SR1) and HsACTN4(SR1). (D) Alignment of SpAin1(SR2) and HsACTN4(SR4).
Supplemental Figure S2. SpAin1 binds F-actin. (A and B) High speed (100,000 x g) sedimentation of 3.0 µM SpAin1 following incubation for 20 min at 25°C with a range of concentrations of F-actin preassembled from 15 µM Mg-ATP actin. (A) Coomassie blue-stained gel of pellets. (B) Plot of the dependence of SpAin1 in the pellet on actin concentration. Error bars, s.e.; n = 3.
Supplemental Figure S3. TMR-labeling of SpAin1 does not affect its bundling ability. (A and B) Low speed (10,000 x g) sedimentation of 5.0 µM Mg-ATP actin following incubation for 20 min at 25°C with a range of concentrations of unlabeled or 33.3% TMR-labeled SpAin1. (A) Coomassie blue-stained gel of supernatants. White and red triangle denote increasing concentrations of unlabeled and TMR-labeled SpAin1. (B) Plot of actin in supernatant over range of concentrations of unlabeled SpAin1 (black circles) or TMR-labeled SpAin1 (red circles). (C-D) Two-color TIRFM of 2.5 µM Mg-ATP actin (10% Oregon Green labeled) and 300 nM SpAin1 (1 or 10% TMR-labeled). (C) Representative fields at similar filament densities. Scale Bar, 5 µm. (D) Quantification of percent of bundled F-actin in the presence of 300 nM SpAin1 (1 or 10% TMR-labeled). Error bars, s.e.; n=2.
**Supplemental Figure S4.** Gel filtration of HsACTN4 and SpAin1. Elution profile of HsACTN4 (left) and SpAin1 (right) from a HiPrep 16/60 Sephacryl S-400 HR size-exclusion column. Coomassie-blue stained SDS-PAGE gels confirm the presence of non-degraded α-actinin in the peak fractions (*).
Supplemental Figure S5. SpAin1 is more dynamic than HsACTN4 on three-filament bundles. (A–C) Two-color TIRFM of 2.5 μM Mg-ATP actin (33.3% Oregon green actin) assembled in the presence of 900 nM SpAin1, HsACTN4 or SpAin1(R216E) (1% TMR-labeled). (A) Merged fluorescent micrographs of α-actinin on three-filament bundles. Scale Bar, 5 μm. (B) Time-lapse fluorescence micrographs of TMR-labeled α-actinin on corresponding bundles in (A). Arrows mark speckles that remain for more (red) or less (green) than 1.5 sec. (C) Kymographs of α-actinin on bundles in (A) over 20 sec. Red boxes mark the 1.5 sec of montages in (B). Scale Bars, 5 μm (horizontal) and 5 sec (vertical). Histograms of residence time, and exponential fits of the fraction of α-actinin bound over time on three-filament bundles is shown in Figure 4D and 4E.
Supplemental Figure S6. Fluorescence intensity correlation of α-actinin speckles on F-actin bundles. Mean fluorescence of α-actinin speckles are plotted against residence time on two-filament (A) or three-filament (B) bundles. Inset: zoomed-in versions showing details of speckles with less than six seconds of residence time.
Supplemental Figure S7. SpAin1(R216) causes ring assembly delays when expressed at low amounts. (A–C) Cytokinesis kinetics of SpAin1 null ain1-ΔI cells expressing either (A) WT SpAin1-GFP or (B) mutant SpAin1(R216E)-GFP from the low strength 8Inmt1 promoter integrated at the ura locus. (A and B) Fluorescent micrograph montages of the time course of Rlc1-tdTomato-labeled contractile ring formation and constriction (right), corresponding to the boxed regions in DIC images (left). Cytokinesis stages are marked with red numbers as indicated in (C). Scale bars, 5 μm. (C) Plot of major cytokinesis events over time. Error bars, s.d.; n ≥ 10 cells. Plots of average duration of contractile ring assembly and constriction are shown in Figure 7, E and F.
Supplemental Figure S8. Mutant 81nmt1-SpAin1(R216E)-GFP exhibits a synthetic interaction with myo2-E1. (A-C) General cytokinesis defects four hours after shifting cells to the semi-restrictive temperature of 28°C. (A) Fluorescent micrographs of myosin-II mutant myo2-E1 cells, SpAin1 null ain1-Δ1 cells expressing mutant SpAin1(R216E)-GFP from the low (81nmt1) strength promoter integrated at the leu locus, and double mutant myo2-E1, ain1-Δ1 81nmt1-SpAin1(R216E)-GFP cells stained with DAPI and Calcofluor to visualize nuclei and septa. Scale bar, 5 µm. (B) Percentage of cells with 1, 2, or >2 nuclei. n≥350 cells. (C) Percentage of normal (n) and abnormal (a) septa. n≥100 septa.
SUPPLEMENTAL VIDEOS

Video 1. Visualization of actin filament bundle polarity. Related to Figure 2A. TIRFM visualization of the addition of 0.75 µM Mg-ATP actin (33.3% Oregon green-labeled) to preassembled F-actin bundles (red boxes) made by incubating crosslinkers with 15 µM F-actin (10% Oregon green-labeled). 100X speed. Scale bar, 5 µm.

Video 2. SpAin1 assembles bundles of mixed polarity. Related to Figure 2B. TIRFM visualization of the addition of 0.75 µM Mg-ATP actin (33.3% Oregon green-labeled) to parallel and anti-parallel two-filament bundles (red boxes), which were preassembled with SpAin1 from 15 µM F-actin (10% Oregon green-labeled). Arrowheads mark elongating barbed ends. 70X speed. Scale bar, 5 µm.

Video 3. Visualization of fluorescently labeled SpAin1-mediated F-actin bundle formation. Related to Figure 3A. Merged two-color TIRFM visualization of the assembly of 2.5 µM Mg-ATP actin (33.3% Oregon green-labeled) (green) in the presence of 300 nM SpAin1 (20% TMR-labeled) (red). Blue and Yellow arrowheads mark elongating barbed ends and SpAin1 decoration. 70X speed. Scale Bar, 5 µm.

Video 4. Visualization of SpAin1- and HsACTN4-mediated F-actin bundle formation. Related to Figure 3B. TIRFM visualization of the assembly of 2.5 µM Mg-ATP actin (33.3% Oregon green-labeled) in the absence or presence of 100 nM HsACTN4 or SpAin1. 100X speed. Scale Bar, 5 µm.

Video 5. Single molecule analysis of fluorescently-labeled SpAin1 and HsACTN4 on two-filament bundles. Related to Figure 4A-C. TIRFM visualization of the dynamics of 900 nM α-actinin (red, first frame; white, subsequent frames) on two-filament F-actin bundles (green, first frame) assembled from 2.5 µM Mg-ATP actin (33.3% Oregon green-labeled). Arrowheads mark α-actinin speckles that remain bound to bundles for more (red) or less (green) than 1.5 sec. 3.5X speed. Scale Bar, 5 µm.

Video 6. Single molecule analysis of fluorescently-labeled SpAin1 and HsACTN4 on three-filament bundles. Related to Supplemental Figure S4A-C. TIRFM visualization of the dynamics of 900 nM α-actinin (red, first frame; white, subsequent frames) on three-filament F-actin bundles (green, first frame) assembled from 2.5 µM Mg-ATP actin (33.3% Oregon green-labeled). Arrowheads mark α-actinin speckles that remain bound to bundles for more (red) or less (green) than 1.5 sec. 3.5X speed. Scale Bar, 5 µm.

Video 7. Visualization of SpAin1(R216E)-mediated F-actin bundle formation. Related to Figure 5C. TIRFM visualization of the assembly of 2.5 µM Mg-ATP actin (33.3% Oregon green-labeled) in the presence of 300 nM SpAin1 SpAin1(R216E). 70X speed. Scale Bar, 5 µm.

Video 8. Visualization of the cytokinesis in S. pombe cells. Related to Figure 7A-D. Fluorescence microscopy of the time-course of contractile ring assembly and constriction of the indicated strains visualized by Rlc1-tdTomato. The last frame of each panel indicates disappearance of Rlc1-tdTomato signal. Blue arrowheads mark ring material unevenly distributed to one side of the division site. Green arrowheads mark excessive ring material. 600X sec. Scale Bar, 5 µm.

Video 9. Monte carlo simulations of search-capture-pull process facilitated and inhibited by crosslinking. Related to Figure 8C-D. Two nodes initially separated by 600 nm polymerize actin at a 15° angle to the horizontal at a rate of 175 nm/sec. (Upper panel) 'Search' is facilitated by a crosslinker attachment rate of 1/sec with an attachment lifetime of 0.3 sec ($K_d^{-1}$=0.3). After capture, myosin walks along the filament with an unloaded rate of 400 nm/sec. (Lower panel) 'Search' is inhibited by excessive bundling from a crosslinker attachment rate of 3.0/sec with an attachment lifetime of 1.0 sec ($K_d^{-1}$=3.0).
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