Dynamic Network Morphology and Tension Buildup in a 3D Model of Cytokinetic Ring Assembly

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ABSTRACT During fission yeast cytokinesis, actin filaments nucleated by cortical formin Cdc12 are captured by myosin motors bound to a band of cortical nodes and bundled by cross-linking proteins. The myosin motors exert forces on the actin filaments, resulting in a net pulling of the nodes into a contractile ring, while cross-linking interactions help align actin filaments and nodes into a single bundle. We used these mechanisms in a three-dimensional computational model of contractile ring assembly, with semiflexible actin filaments growing from formins at cortical nodes, capturing of filaments by neighboring nodes, and cross-linking among filaments through attractive interactions. The model was used to predict profiles of actin filament density at the cell cortex, morphologies of condensing node-filament networks, and regimes of cortical tension by varying the node pulling force and strength of cross-linking among actin filaments. Results show that cross-linking interactions can lead to confinement of actin filaments at the simulated cortical boundary. We show that the ring-formation region in parameter space lies close to regions leading to clumps, meshworks or double rings, and stars/cables. Since boundaries between regions are not sharp, transient structures that resemble clumps, stars, and meshworks can appear in the process of ring assembly. These results are consistent with prior experiments with mutations in actin-filament turnover regulators, myosin motor activity, and changes in the concentration of cross-linkers that alter the morphology of the condensing network. Transient star shapes appear in some simulations, and these morphologies offer an explanation for star structures observed in prior experimental images. Finally, we quantify tension along actin filaments and forces on nodes during ring assembly and show that the mechanisms describing ring assembly can also drive ring constriction once the ring is formed.

INTRODUCTION

Cytokinesis in fungi and animal cells requires self-organization of a medial contractile ring of dynamic actin filaments, myosin motors, and associated proteins to cleave a mother cell into two daughter cells (1–3). In the fission yeast Schizosaccharomyces pombe, a model organism for the study of cytokinesis, the cytokinetic ring assembles from a broad band of membrane-associated node complexes, which bind myosin II molecular motors and Cdc12 formins. The nodes condense into a narrow ring in a process that depends on actin polymerization and lasts ~10 min (4,5). In early studies using fixed cells to study the formation of the fission-yeast contractile ring in three dimensions (3D), actin filaments were found to organize in starlike bundles (or cables) near the cellular membrane, suggestive of a leading-cable mechanism for ring assembly (6) (Fig. 1 A). In this picture, the ring assembles through circumferential elongation of actin cables in opposite directions and closure of the cable structure (7). Later studies using 3D time-lapse images of live cells indicated a dynamic and uniform actin-filament network in the medial cell cortex, supporting a picture of a fluctuating and distributed network of filaments that polymerize and depolymerize at multiple locations around the cell middle (8,9).

Consistent with a process of ring assembly through a broad condensing actomyosin network, changes in the dynamics of actin filaments during ring formation lead to delayed ring assembly or formation of nonfunctional actomyosin structures, such as clumps, extended meshworks, and double rings. Increase in the concentration of actin-filament cross-linkers α-actinin Ain1 and fimbrin Fim1 leads to extended actomyosin meshworks, whereas deletion of Ain1 with simultaneous reduction of Fim1 concentration leads to clumps (10) (Fig. 1 B). Mutations that impair Cdc12-mediated actin polymerization lead to node clumps (11,12). In cells where the cofillin Cof1 severing activity is compromised by mutations, nodes coalesce into multiple clumps instead of a ring (13).

The interplay between actin-filament polymerization, myosin motor activity, severing, and cross-linking interactions in the morphology of the condensing actin network has been previously characterized theoretically using a computational model (search, capture, pull, and release (SCPR)) (8,10). This model simulated the polymerization of actin filaments along random directions out of formins at nodes and the establishment of transient contractile actomyosin connections among neighboring nodes. It recapitulated the stochastic start-stop motion of cortical nodes...
mostly toward, but also away from, the cell middle (8). The model also described the alignment of nodes during ring assembly, as well as clump and meshwork formations after varying the degree of cross-linking interactions among simulated filaments (10). However, whether the cable-star structures initially observed in Arai and Mabuchi (6) can arise through an SCPR mechanism has not been addressed. One of the aims of this study is to test whether a 3D model based on the SCPR mechanisms can also lead to star/cable morphologies during contractile ring assembly.

The previous SCPR models (8,10) were developed in two dimensions (2D) representing the cortical cell membrane surface, where membrane-bound node component Mid1 accumulates in nodes and helps recruit myosin Myo2 and Cdc12 (14). However, how the actin filaments that reach 0.5–2 μm in length become parallel and tightly packed along the cell membrane has not been resolved. It has been proposed that the cortical endoplasmic reticulum (ER) that influences Mid1 distribution (15) may also confine actin filaments near the membrane by restricting their motion (2). Alternatively, binding of actin filaments to Myo2, as well as to one another, through cross-linkers, could be a mechanism for confinement. The second aim of this study is to use a 3D model to predict profiles of cortical density of the actin filaments under different conditions of myosin and cross-linker activities and to relate them to the resulting network morphologies.

Prior modeling did not address the buildup of cortical tension during cytokinetic ring assembly. The magnitude and direction of the microscopic tensions acting on filaments and cortical node complexes depends on the morphology of the condensing actin-filament network and the activity of regulators of actin dynamics such as myosin pulling and cross-linking. The third aim of this study is to identify patterns in the evolution of microscopic forces during contractile ring assembly and to relate them to network architecture.

To achieve the above-mentioned objectives, we extend the previous 2D SCPR model of cytokinetic ring assembly (10) to 3D (Fig. 1 C). Results from the 3D model show that: 1), the model parameter space leading to final ring morphologies is near parameter regions that result in cables/stars, networks, and clumps; 2), confinement of actin filaments against the cell cortex can occur just by filament turnover, myosin capturing, and cross-linking interactions, with no need for additional mechanisms, such as confinement by the ER or adhesion of actin filaments at the membrane; 3), in early stages of ring formation, forces acting on the cortical nodes help narrow the broad band, whereas at later stages, they reorient toward the cell interior and can subsequently aid in ring constriction.

**COMPUTATIONAL MODEL**

We extended a prior 2D model (10) using methods developed to study actin cable formation in nondividing yeast (16) (see Fig. 1 C and Appendix). The computational domain was a tube with radius $R = 1.74 \mu m$ and length $13 \mu m$, as in dividing fission yeast. Sixty-five nodes were initially distributed on the cylindrical boundary, randomly circumferentially and according to a Gaussian distribution with standard deviation $\sigma = 0.9 \mu m$ along the cell long axis (8,17,18). Actin filaments were simulated as beads connected by springs growing from two formin dimers (19,20) at the node complexes. Their initial length was three beads. The myosin II motor activity at the nodes was simulated by assigning to each node the ability to capture and pull actin-filament beads that came closer than $r_c = 0.1 \mu m$ (10). This reproduces the power-stroke movement of myosin II.
multiple myosin heads extending out of the nodes with their tails bound to the node complex (14).

Cross-linking was simulated as an isotropic elastic attraction between filament beads with spring constant $k_{\text{crslnk}}$ when beads are closer than a threshold distance, $r_{\text{crslnk}}$. This is a coarse-grained representation of the interactions between actin filaments due to $\alpha$-actinin Ain1 and fimbrin Fim1 (10,21–24). Parameters $r_{\text{crslnk}}$ and $k_{\text{crslnk}}$ implicitly represent the binding/unbinding kinetics of the cross-linkers and their concentrations. The equilibrium distance between two cross-linked actin-filament beads was 0.03 $\mu$m, on the order of the size of Ain1 (10).

To simulate actin-filament turnover due to cofillin severing and Cdc12 turnover, whole filaments were removed at a rate that gives an average filament lifetime of 15 s (8). Upon filament removal, a new filament was allowed to grow from the formin nucleator along a new random direction. To limit the magnitude of pulling forces when nodes connected with bundles of actin filaments, we assumed that the pulling force was reduced by a factor depending on the number of cross-linking interactions of the captured bead with other filament beads (10). Langevin dynamics was used to update the positions of actin filaments and nodes.

RESULTS

The 3D model reproduces cytokinetic ring assembly through intermittent motion of nodes

The 3D model reproduced assembly of a narrow ring of actin filaments and nodes, through condensation of nodes initially distributed within a broad band of width 1.8 $\mu$m (two times the standard deviation of node distribution), along a cylindrical surface (Fig. 2, A and B, and Movies S1 and S2 in the Supporting Material). This was achieved after using parameters from experiment (see Appendix and Table S1) and appropriate choice of cross-linking parameters, as discussed in the next sections.

FIGURE 2 The model reproduces ring assembly through intermittent motion of nodes. See main text and Table S1 for model parameter values. (A) Representative simulation results in top and cross-sectional views (radius, 1.74 $\mu$m) of cortical nodes (red) and actin filaments (dark green) at 0, 60, 300, and 500 s. (B) Width of a broad band of nodes versus time ($n = 12$ simulations, error bar indicates the mean ± SE). The average broad band width at 480 s includes lagging nodes and tilted rings and is wider than the width of the main ring bundle in A. (C) Histograms of node velocity distributions at early, mid-, and late ring assembly ($n = 5$ simulations). Velocities were calculated by measuring displacement over 1 s. (D) Histogram showing distribution of cortical node directions relative to the long cell axis at early, mid-, and late ring assembly ($n = 5$ simulations). The cartoon shows how the angle is computed, where $p_1$ and $p_2$ are consecutive positions of a node moving toward the cell equator. (E) Graph showing start-stop node motion. Individual node motion in different simulations was discretized into a series of moves (velocity > 1 nm/s) and pauses (velocity < 1 nm/s). Upper and lower horizontal lines represent moves and pauses, respectively, and vertical lines indicate state switching. Velocities were calculated by measuring displacement over 5 s. To see this figure in color, go online.
We verified that our model reproduces the features of prior 2D models based on the SCPR mechanism (8,10) that had been used to compare these models against experimental observations. For the parameter set in Fig. 1, the broad band of nodes condensed in ~10 min, consistent with experimental observations (8,10). The distribution of node velocities at the onset of ring assembly (1 min) ranged between 0 and 40 nm/s, as in prior experiments (8,10). A connection between two nodes by a single filament corresponds to 10 nm/s so this distribution reflects nodes connected with one another through variable numbers of actin filaments. The average node velocity decreases with time (Fig. 2 C), a result of 1), cancelation of forces pulling in opposite directions circumferentially; 2), an increase in excluded-volume interactions that prevent node overlap; and 3), an increase in cross-linking interactions among filaments, which we assume result in a reduction of myosin pulling (see Appendix).

The distribution of node movement direction in simulations was broad, as observed (8,10) (Fig. 2 D): at the onset of ring assembly, ~50% of the nodes moved within 30° of the cell long axis (Fig. 2 D, inset); at later stages of ring assembly, ~40% of the nodes moved within 30° of the cell long axis, indicating an increase in circumferential movement of the nodes along the cortex, consistent with results from prior experiments (10).

The 3D model also reproduced features of node motion in experiments, which is characterized by many start-stops and changes of direction of motion (8,10). Fig. 2 E shows node intervals of movement between pauses. The probability for a node to change direction by >15° after a pause was >50% at all times of ring assembly. The frequency of node start-stops increased with the progression of ring assembly, reaching a plateau at 150–300 s (data not shown).

**Actin filament confinement against the cortical membrane**

The model results show that cross-linking interactions among filaments contribute to their localization close to the cell membrane. In simulations using the same parameters as Fig. 2, actin filaments grew out of nodes along random directions, got cross-linked to one another, and were captured and pulled toward the cortex by nodes. Snapshots showing cross-sectional views of the computational domain are shown in Fig. 3 A. This figure shows that filaments spanning the cell cross section during ring assembly were mostly unbundled. Future experiments could test the model predictions by imaging actin filaments growing toward the cell center and comparing the images to simulated 3D micrographs (Fig. S1).

Profiles of actin-filament bead density within 0.174 μm of the cortical membrane, equal to 10% of the cell radius, showed that the confinement process occurs progressively over several minutes (Fig. 3, B and C). At the onset of ring assembly, >90% of beads were within 0.174 μm of the cell cortex, since the simulations started with three beads per filament. Since the average lifetime of each filament was 15 s, the filament length distribution equilibrated within 1 min. By that time, ~80% of the filament beads were within 0.174 μm of the cortex, and this proportion increases to >90% at later stages of ring assembly. This indicates a cooperative process in which the probability of a filament growing toward the cell center to realign along the cell surface increases with time, due to the increasing fraction of confined filaments.

![Figure 3](image-url)
The alignment of filaments into a bundle along the 2D cortex is similar to the mechanism of confinement in the radial direction (Fig. 3 D). The initial direction of filament elongation was random, but by 1 min, most of the actin filaments reoriented parallel to the cell long axis, with ~70% having angles of <30° with the cell long axis. With the progression of ring assembly, actin filaments reoriented perpendicular to the cell long axis, with ~50% forming angles of 30° with the long axis (Fig. 3 D). The direction of filament elongation with respect to the equator (location of ring) also changed with time (Fig. 3 E). After the first minute, approximately half of the actin filaments elongated toward the cell equator (equatorial filaments) and half toward the cell poles (polar filaments; see Fig. 3 E). The fraction of equatorially growing filaments increased with time (Fig. 3 E). The overall broad distribution of actin-filament orientations is consistent with prior experimental measurements (9).

Network morphology as a function of cross-linking properties

We next focused on how actin-filament cross-linkers contribute to the organization of actin filaments and nodes in a bundle on the cell cortex. We used a set of three parameters, width of the node broad band, percent of actin filaments close to the membrane, and 1D porosity, to quantify the morphology of node-filament structures that form when parameter values of the simulations are varied, similar to prior work (10,25). The node band width was computed from the standard deviation, σ, of node positions along the cell long axis as \( w = 2\sigma \). The 1D porosity, a measure of the uniformity of node distribution along the circumferential direction, was computed as the ratio of the sum of the lengths of all circumferential gaps with no nodes to the cell circumference, \( 2\pi R \).

The above three measures, together with visual inspection of the results, indicate regions in parameter space that provide different node-filament morphologies after 500 s of simulation time. Fig. 4 shows results using the same parameters as in previous figures, but varying cross-linking parameters \( r_\text{crslnk} \) and \( k_\text{crslnk} \). The latter are effective parameters that represent the combined action of the multiple cross-linkers. The cross-link formation rate depends on \( r_\text{crslnk} \), (thus, larger cross-linker concentration corresponds to larger \( r_\text{crslnk} \),) and the rate of cross-link breakage depends on both \( f_\text{crslnk} \) and \( k_\text{crslnk} \) (breakage rate decreases as either parameter increases). When both \( r_\text{crslnk} \) and \( k_\text{crslnk} \) were small compared to the other values tested in Fig. 4, the broad band of nodes did not condense toward the cell middle (\( w \) remained >1.2 \( \mu \)m) but instead organized into many small clumps. In this case, cross-linking is too weak to

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confine actin filaments along the cortex, and thus, nodes establish few connections with their nearest neighbors, which results in many clumps (26).

In the region marked few clumps (Fig. 4 D), \( k_{\text{crslnk}} \) is high enough to allow partial confinement of actin filaments and condensation; however, overall cross-linking is still too weak to align nodes and filaments into a bundle, which results in few clumps. Using \( r_{\text{crslnk}} \approx 0.12 \mu m \) and \( k_{\text{crslnk}} \approx 1\) pN/\( \mu m \) results in ring formation. The results shown in Figs. 2 and 3 were located in this region. For \( r_{\text{crslnk}} >0.15 \mu m \) and \( k_{\text{crslnk}} \approx 1\) pN/\( \mu m \), premature formation of bundles lead to meshworks, which often consisted of double rings. This transition from clumps to rings to meshworks for \( k_{\text{crslnk}} \approx 1\) pN/\( \mu m \) is similar to the results of the 2D model of Laporte et al. (10) and experimental observations of cells with varying concentrations of cross-linkers (10). However, here we show that this occurs when parameters allow partial filament confinement on the cell membrane and that this confinement is weaker in the clump region.

In two regions of parameter space of Fig. 4, the morphology of the aggregates resembles that of stars, which are transient structures that last 1–5 min. The main difference between clumps and stars is in the organization of the actin filaments: in both cases, nodes coalesce into disconnected aggregates, but in stars, the actin filaments elongating from the node aggregates formed several bundles of aligned filaments that extended along the cell cortex. The few-clumps region also showed a slightly higher porosity with respect to that of stars (Fig. 4 C).

For the highest cross-linking parameters in the simulations, only one or two bundles emerge from the node aggregates, resulting in configurations that resemble actin cables. The cables align along the long axis of the cell such that they do not bend during extension. This phase occurred with both high \( k_{\text{crslnk}} (>1\) pN/\( \mu m \)) and high \( r_{\text{crslnk}} >0.12 \mu m \). Cables had the maximum density of actin filaments at the cortex (Fig. 4 B) and the maximum circumferential porosity (Fig. 4 C) with respect to the other simulated actomyosin morphologies.

**Impact of myosin motor pulling force on network morphology**

Myosin motor pulling works together with actin-filament cross-linking to assemble the contractile ring (10). To explore this interplay in 3D, we varied the force per node-filament connection described by parameter \( F^0_{\text{myo}} \) together with one of the two parameters describing the strength of cross-linking interactions, \( r_{\text{crslnk}} \). For Fig. 5, we used the same three measures as for Fig. 4 to quantify the resulting morphology after 500 s of simulation time.

A minimum amount of myosin pulling was necessary to condense the broad band of myosin nodes toward the cell equator, corresponding to \( F^0_{\text{myo}} \approx 1\) pN (Fig. 5 A). This is consistent with the experimental and modeling results in (10) that showed failure of node condensation in myo2-E1 mutant cells with defective Myo2 motor activity. For such cases, the resulting configuration is an extended meshwork structure, as in Fig. 5 E, with a varying degree of actin-filament confinement along the surface depending on the value of \( r_{\text{crslnk}} \).

We find that the region of ring formation lies approximately along the diagonal in the parameter space of Fig. 5 D. Instead of rings, weak cross-linking interactions lead to either a few node clumps or else to many small clumps if the node pulling force is large enough (Fig. 5 D, upper left). Sufficiently high cross-linking interactions lead to extended node-filament meshworks instead of rings, which is a result of our assumption that the node pulling force per captured filament decreases with increasing number of cross-links. As cross-linking parameter \( r_{\text{crslnk}} \) increases to \( >0.11\) \( \mu m \), a higher value of pulling force \( F^0_{\text{myo}} \) is required for ring formation, to overcome the slowing down induced by the cross-linking. These results are in agreement with the results in Laporte et al. (10), both with the 2D model in this work and with the experimental observations that 1), Myo2 overexpression rescues the meshwork phenotype of Ain1-overexpressing cells, and 2), Myo2 overexpression results in clumps in cells with deleted Ain1. These results also show that the magnitude of the myosin pulling force...
provides an additional parameter that cells can use to achieve ring formation, in addition to tuning cross-linking parameters (Fig. 4 D).

**Buildup of tension and filament polarity during cytokinetic ring assembly**

To examine how tension builds up for ring constriction during the assembly of the contractile ring, we first studied the tension along actin filaments (Fig. 6A, color map, and Fig. S2). Microscopic tension between consecutive beads of an actin filament was positive or negative depending on the extension or contraction of the spring connecting the filament beads. Thermal forces produce tension between consecutive filament beads of average magnitude ~0.6 pN, using spring constant 100 pN/μm between filament beads. Compressive forces between actin-filament beads arise from formin-induced polymerization and extensive forces from myosin pulling (4 pN for a single captured filament). Cross-link formation and breakage also contributes to the spatially varying tension distribution shown in Fig. 6A, where a few spots of contractile force >1 pN can be seen at all stages of ring assembly. On average, actin-filament springs are extended, as shown in Fig. 6B. The forces on nodes are shown as vectors (Fig. 6A, arrows). These forces became constricting during ring assembly, pointing toward the cell center (Fig. 6A).

Nodes pull on actin filaments that form a bundle of filaments of mixed polarity. This is shown in Fig. 6C, where the actin filaments with a barbed-to-pointed end orientation in the clockwise direction around the cell axis are shown in red, and those in the counterclockwise direction in blue.

FIGURE 6 Buildup of tension during ring assembly and polarity of filaments in a simulated contractile ring. (A) Snapshots of simulations at early and late stages of ring assembly, showing actin-filament tension distribution, with colors representing the spring force between actin-filament beads. Black segments are springs connecting formins to nodes. Positive and negative values correspond to extension and compression, respectively. Arrows indicate the sum of all forces acting on nodes (red circles), except boundary force. Node forces become constricting with increasing time. The magnitude of the largest vector is 11 pN. (B) Average actin-filament bead spring force over time, computed from seven independent simulations. (C) Snapshots of simulations showing assembly of a ring of filaments with mixed polarity. Nodes are yellow and Filaments are red or blue, depending on their clockwise or counterclockwise barbed- to pointed-end orientation around the cell long axis. (D) Contractile ring tension (see main text for definition) versus time. Values are presented as the mean ± SE from seven independent simulations. At 600 s, the force that restricts nodes to the cell boundary is set to zero and the ring starts to constrict. (E) Snapshots of the constricting ring obtained by setting the force that restricts nodes to lie on the cell boundary to zero. (F) Results of a simulation with a cell forming a transient star and filaments colored according to orientation as in panel C. A transient star structure is observed at 200 s, which eventually transforms to a ring. At 500 s, filaments form polarized parallel bundles out of a node clump. As the ring closes, the clump disperses and filament polarity becomes mixed around the ring. Results in all cases show simulations with the same parameter values as in Fig. 1 and Table S1. To see this figure in color, go online.
The development of a bundle of mixed filament polarity depends on filament growth along random directions and intermediate cross-linking strength that allows filaments to align and slide past one another as they polymerize (Fig. 4). For the reference parameter values (Table S1), the average ratio of clockwise- to counterclockwise-oriented filaments in the fully assembled ring was 1:1 and remained approximately uniform around the ring. We calculated the total circumferential tension for the reference model parameter values, which increases over time, reaching a value of ~4 pN (Fig. 4 D).

The contractile rings of our 3D model constrict if we eliminate the force that keeps the nodes on the boundary after ring assembly (at 600 s). This is shown in Fig. 6 E and Movie S3, where the nodes were released from the boundary but their drag coefficient was unchanged. When nodes were released from the cell boundary, the ring tension increased as the average distance between nodes decreased and the overlap between filaments and nodes increased (Fig. 6 D).

Without any modification to our model, the resulting rate of ring constriction, ~0.2 μm/min, is of the same order as the experimentally measured value, 0.3 μm/min (27). We note, however, that this agreement may be the result of underestimating both tension and drag, since the measured tension of contractile rings of fission yeast protoplasts is ~390 pN, working against an estimated drag coefficient 55 times larger than the drag due to the precursor nodes (27) (see Discussion).

Even for the reference parameter values, we occasionally observed transient star shapes that became rings, as can be seen in Fig. 6 F and Movie S4) (9). Fig. 6 F shows two polarized bundles that emanate from a former star-shaped structure (red and blue bundles at t = 500 s). These bundles connected with other linear structures and formed a ring, which developed into a bundle of filaments of mixed polarity.

**DISCUSSION**

In this work, we presented a 3D model of contractile ring assembly based on the SCPR mechanism. Our model predicts that confinement of actin filaments against the cell cortex can occur just by filament turnover and cross-linking interactions, with no need for additional mechanisms, such as confinement by the ER or adhesion of actin filaments at the membrane. However, we cannot exclude the possibility that additional confining mechanisms contribute to the robustness of ring assembly by enlarging the region of parameter space where rings can form. The excluded volume of the nucleus, which is located in the middle of the cell at the onset of actin polymerization at the nodes, can play some role in the early stages of ring assembly, which is the stage with the largest fraction of unconfined filaments in the simulations (Fig. 3, B and C). Experiments featuring imaging of actin-filament dynamics in 3D can elucidate these aspects. More generally, our 3D model can be related to the dynamics of actin-filament confinement at the cortex of nondividing cells, where formins, actin filaments, crosslinkers, and myosin motors localize close to the plasma membrane (28–30).

Our work provides general predictions about the morphodynamic landscape of the node-filament system during ring assembly, as a function of myosin and cross-linker activities. We found that in the parameter space of the model, the region of ring assembly is located between regions where actin filament and nodes coalesce into clumps, meshworks/double rings, or stars. This structure of different morphologies in parameter space likely represents a general feature of the node-filament system, resulting from the underlying mechanisms of tethered actin-filament polymerization, actin-filament turnover, transient actomyosin connections, and cross-linking among actin filaments. It may not be possible to change all the model’s parameter values independent of one another using mutations and pharmacological treatments in experiments. However, any such perturbations that influence the microscopic mechanisms implemented in the model (polymerization, cross-linking, and myosin pulling) should shift the system toward the different morphological regions in a continuous manner. These large-scale changes in structure are some of the most reliable predictions of the model, since they are less dependent on the precise choice of parameter values.

Results from our 3D model of contractile ring assembly also showed that the boundaries between different morphological regions are not very sharp (the system is finite in size, so fluctuations are significant). For example, rings occasionally form in the star region of parameter space and vice versa. An interesting possibility for further study is to explore whether our model correctly captures the magnitude of fluctuations in the system. In our model, fluctuations arise from the random placement of nodes on the cell boundary, the randomness in actin-filament growth direction, and thermal forces. It is possible that the cells experience even larger fluctuations (for example, from big pieces of cables that get into the ring, nonuniform Cdc12 clustering (31), activation signals, or outward pulling by myosin V motors). If such mechanisms work against reliable ring assembly, additional control mechanisms or dynamical pathways may have evolved to deal with such aspects.

Dynamic cross-linking in our model of ring assembly allows polymerizing filaments to align and form bundles of filaments of mixed polarity. When using values of cross-linking parameters that result in long-lived cross-links, we obtained star and cable structures, resulting from buckling of filaments polymerizing toward one another and formation of bundles of filaments with the same polarity. These results illustrate how bundles of different polarity can form, depending on filament nucleation geometry and cross-linking dynamics (10,16,32).
Analysis of contractile rings in electron micrographs of cdc25-22 cells at early stages of ring assembly showed segregated filament polarity, in support of the leading actin cable model for ring assembly (7). A limitation of that model (7) is the difficulty of measuring actin-filament orientation in electron micrographs. The results from our 3D model (Fig. 6 F) offer an alternative explanation as to how such assembly can develop in wild-type or mutant cells, without assuming that ring formation starts from a single spot.

In this work, we emphasized the effects of cross-linking and myosin pulling. Decrease in node number and reduction of filament length (by either a decrease of polymerization rate or an increase in turnover) enlarge the region of clump formation (8,13,26). Since the nodes are an important component of this model, slow ring assembly in cells that lack nodes (33) will have different features from those presented here. Future modeling work based on our 3D model could incorporate cytoplasmic filament nucleation (12,34) and actin cable cortical flow toward the cell middle, the relative contribution of which has been debated (12,35).

Results from the simulations of ring constriction in Fig. 6 provide additional support for the mechanisms of fission yeast ring constriction proposed in Stachowiak et al. (27), where, in a 2D model similar to ours, formins polymerized semiflexible actin filaments, myosin motors were able to capture and pull the filaments, and turnover of actin filaments was incorporated. The following assumptions were different in the Stachowiak model (27): 1), formin nucleators were not linked to the node complexes; 2), both the total myosin pulling forces and the total drag experienced by the ring were larger by 1–2 orders of magnitude; and 3), the density of ring components, including formins and myosin motors, decreased during constriction (36). Together, the prior study (27) and the present work suggest that mechanisms similar to those that drive ring assembly also drive ring constriction, although formin anchoring and node composition may be modified during the ring maturation phase, perhaps for better control or in response to a higher load during constriction. This constriction mechanism that depends crucially on anchored formin polymerization, dynamic actin-filament cross-linking, and actin-filament turnover differs from that of other models where contractility relies on tension generation by actin-filament depolymerization and cross-linking (37–39), myosin-induced depolymerization (40), or contractile units (41). It is also different from contraction mechanisms due to nonlinear actin-filament elasticity (42,43) or myosin filament rotation (44) in bundles without actin-filament turnover.

Our 3D model for fission yeast contractile ring assembly may have implications in the cytokinesis of larger animal cells, which also rely on related mechanisms involving formins, myosin II, actin-filament polymerization, and cross-linking (45–47). A continuum theoretical treatment, averaging over individual filaments and motors to describe the properties of the actomyosin cortex is appropriate for these larger systems (48–51). Continuum models implementing hydrodynamic equations that account for membrane curvature and filament orientation were also proposed to predict morphologies such as stars, cables, stationary rings, and moving rings, and it has been shown that curvature-orientation coupling impacts network morphology (49,52). However, this study shows that morphological transitions can be largely controlled from filaments, cross-linkers, and motors at the micrometer scale. Future modeling studies examining this crossover of scales together with systematic in vitro experiments (53,54) should help clarify the link between microscopic dynamics and large-scale cytoskeletal structures.

**APPENDIX: COMPUTATIONAL METHODS**

### Actin filament representation

Actin filaments were represented by point beads connected by harmonic springs (55–57). Langevin dynamics was used to update the positions $r_i$ of the $i^{th}$ actin-filament bead in simulation time $dt$:

$$ F^{spring}_i + F^{bend}_i + F^{thermal}_i + F^{crslnk}_i + F^{myo}_i + F^{spt}_i = \zeta_b dr_i / dt, \quad (1) $$

where $\zeta_b = 0.108$ pN s/µm is an effective drag coefficient of the filament segment (10), assumed equal in the three directions, and the forces are defined below. The elastic spring force between consecutive beads was $F^{spring}_i = -\partial E^{spring} / \partial r_i = -(k/2) \sum_{j>i} (|r_{i+j}-r_i| - l_0)^2 / |r_{i+j}-r_i|$, where $l_0 = 0.1$ µm is the equilibrium length representing 37 actin subunits. This scale is small enough to include molecular-level details, such as myosin capturing from nodes or cross-linking interactions of nearby actin-filament beads, but also smaller than the typical filament length and cell size that are on the order of micrometers. The spring constant between consecutive beads was $k = 100$ pN/µm, a value that maintains the segment length between adjacent beads approximately constant for $dt$ as large as $3 \times 10^{-4}$ s (10,58). To reproduce the bending rigidity of the actin filaments, the bending force was computed as $F^{bend}_i = -\partial E^{bend} / \partial r_i = -(k_B T \zeta_0 / l_p) \sum_{j>i} r_{i+j} \cdot t_{i+j} / |r_{i+j}|$, where $t_{i+j} = (r_{i+j} - r_i) / |r_{i+j} - r_i|$, is the tangent vector and $l_p = 10$ µm is the persistence length (59). The thermal force obeys: $F^{thermal}_i = \zeta B \cdot \partial E^{thermal}_i / \partial r_i$, where $B$ is the second-order unit tensor (55). The filament model used here was validated previously (16) by testing that it reproduces actin-filament mechanical and dynamic properties, such as persistence length, relaxation dynamics, and energy equipartition among modes.

### Cross-linking interactions between actin filaments

When bead $i$ of one filament was within $r_{crslnk} = 0.12$ µm of the closest bead $j$ of another filament, the force in Eq. 1 was $F^{crslnk}_i = -(k_{crslnk}/2) \sum_{j>i} (|r_{i+j} - r_i| - r_0)^2 / |r_{i+j} - r_i|$, Parameter $r_0 = 0.03$ µm is the equilibrium distance between two cross-linked actin filament beads.

### Actin filament polymerization

The presence of $\sim 4$–6 Cdc12 formins per node (12,36) was simulated by assuming two active formin dimers connected to each node through elastic springs of stiffness $k_{Cdc12} = 100$ pN/µm and equilibrium length $r_{Cdc12} = 0.1$ µm. Filament polymerization from nodes was simulated by increasing

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the equilibrium length of the spring that connects the formin bead with the first filament bead. The polymerization rate was \( v_{pol} = 0.1 \text{ m/s} \) (9). When the length of the growing segment was larger than two times \( l_0 \), a new bead was introduced. We assumed that an actin filament can rotate freely around the nodes, i.e., that their attachment to the nodes through Cdc12 responds to a flexible hinge. We anticipated that our results would not depend on this assumption (10).

**Node capturing, pulling, and motion**

Capturing of actin-filament beads that passed within 0.1 \( \mu \text{m} \) from a node was established by a harmonic interaction potential with spring constant 2.5 pN/\( \mu \text{m} \) and equilibrium length 0 \( \mu \text{m} \). Upon establishing a connection, the node exerted an additional tangential pulling force on the filament bead. \( F_{myo} \approx 4 \text{ pN} \), toward the barbed end of the captured filament. To satisfy force balance, an equal and opposite force was exerted on the connected node. Nodes could establish only one connection with the same filament but were able to connect with many filaments. To limit the magnitude of pulling force when nodes connect with bundles of actin filaments, the pulling force on each connected bead was \( F_{myo} = \frac{\mu F_{pol}}{N_s^2}, \) where \( \mu = 0.3 \) and \( N_s \) is equal to the number of filament beads cross-linked to the captured bead, when \( N_s > 3 \). This reduction in force represents the distribution of myosin force over many filaments in the bundle and the interference of myosin activity with actin cross-linkers. Here, we used a stronger dependence of \( F_{myo} \) on \( N_s \) compared to that used in Laporte et al. (10), but our results are not influenced by this choice. To avoid node overlap, nodes were subjected to exclude volume interactions: when the centers of two nodes were <0.2 \( \mu \text{m} \) from each other, a repulsive radial force of magnitude 50 pN was applied. The position of a node, \( r_{node} \), was found by solving \( \frac{dt_{node}}{dt} = \frac{F_{rad}}{r_{node}}, \) where the node drag coefficient was \( \sigma_{node} \approx 400 \text{ pN s/\mu m} \) (8). The total force on the node, \( F_{rad} \), is the sum of 1), elastic forces transmitted through filaments polymerizing out of the node; 2), forces due to the elastic spring that connects the node captured actin-filament beads; 3), forces that balance node pulling on actin filaments; and 4), excluded-volume interactions.

**Boundary conditions**

Nodes remained bound to the cell cortex via a confining force of 5 pN toward the cell boundary. Actin filaments polymerized from formins were prevented from crossing the domain boundary by applying an inward force of magnitude 5 pN normal to the boundary on each bead crossing it.

**Calculation of circumferential tension**

We calculated the total circumferential tension as done in Stachowiak et al. (27) by summing the component of tension in the circumferential direction from tension-bearing springs, \( T = \sum \cos^2(\theta_j)l_j/(2\pi R), \) where, \( T, l_j, \) and \( \theta_j \) are the tension, length, and angle of spring \( j \) with respect to the ring plane. In the sum, we included all springs between filament beads and all cross-linker springs, which are the main contributors to \( T \).

**Comparison to previous models based on SCPR**

The main differences of this 3D model compared to the earlier 2D models of cytokinetic ring assembly are as follows. In Vavylonis et al. (8), actin filaments were represented as growing straight lines, and it was assumed that their polymerization stops when a connection between two nodes is established. That model (8) predicted node clumps and rings, whose asymptotic dependence on parameter values was examined in Okjic and Vavylonis (26). These models did not examine structures such as meshes, stars, and cables. The formation of meshes through local node alignment was examined in Okjic et al. (25). Unlike the models of prior studies (6,25), which focused on the kinetics of node aggregation and treated actin filament as straight lines, the model of Laporte et al. (10) used semiflexible actin filaments. Their results (10) suggested that cross-linking interactions can provide a node aligning force in addition to bundling actin filaments into the contractile ring. Because of the stabilizing effect of cross-linking, the assumption of force-induced reduction of polymerization rate of the original SCPR model was not as important in preventing clumps. Here, we incorporated the same physical mechanisms as in the model of Laporte et al. (10) to investigate ring assembly in 3D and detected more network morphologies.

**SUPPORTING MATERIAL**

Two figures, one table, and four movies are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495(14)01117-5](http://www.biophysj.org/biophysj/supplemental/S0006-3495(14)01117-5).

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**SUPPORTING CITATIONS**

Reference (60) appears in the Supporting Material.

**REFERENCES**


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