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INSIGHTS INTO THE MECHANICS OF CYTOKINETIC RING ASSEMBLY USING 3D MODELING

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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. The myosin motors exert forces that pull nodes together into a contractile ring. Cross-linking interactions help align actin filaments and nodes into a single bundle. Mutations in the myosin motor domain and changes in the concentration of cross-linkers alpha-actinin and fimbrin alter the morphology of the condensing network, leading to clumps, rings or extended meshworks. How the contractile tension developing during ring formation depends on the interplay between network morphology, myosin motor activity, cross-linking and actin filament turnover remains to be elucidated. We addressed this question using a 3D computational model in which semiflexible actin filaments (represented as beads connected by springs) grow from formins, can be captured by myosin in neighboring nodes, and get cross-linked with one another through an attractive interaction. We identify regimes of tension generation between connected nodes under a wide set of conditions regarding myosin dynamics and strength of cross-linking between actin filaments. We find conditions that maximize circumferential tension, correlate them with network morphology and propose experiments to test these predictions. This work addresses “Morphogenesis of soft and living matter” using computational modeling to simulate cytokinetic ring assembly from the key molecular mechanisms of viscoelastic cross-linked actin networks that include active molecular motors.

METHOD

Contractile ring assembly is simulated within a 3D domain that matches fission yeast in shape and dimensions, generalizing a previous 2D model (1, 2), see Fig. 1A. 65 nodes that contain Cdc12 and myosin motors are placed on the cell boundary, within a band at the cell middle (3). Actin filaments are represented as beads connected by springs (Fig 1A). They polymerize at $0.1 \mu\text{m/s}$ out of Cdc12 (two filaments per node). Actin filament turnover due to severing and Cdc12 dissociation from nodes is simulated by randomly removing filaments, with average lifetime 15 s. Cross-linking between filaments is simulated by an

attractive interaction between filament beads closer than $r_c=0.1 \mu\text{m}$ (spring constant $1.5 \text{ pN}/\mu\text{m}$; equilibrium length $0.03 \mu\text{m}$). Beads of actin filaments that approach neighboring nodes closer than $0.1 \mu\text{m}$ establish connections with them, simulated as an elastic interaction (spring constant $2.5 \text{ pN}/\mu\text{m}$). During such a connection, the node exerts an additional pulling force on the bead, $F_{\text{pull}} = 4 \text{ pN}$, towards the barbed end (1, 2), see Fig. 1A. An equal and opposite force is exerted on the connected node. For nodes capturing filament beads already cross-linked, the magnitude of the pulling forces is reduced in proportion to the number of cross-linked filaments (2). Brownian Dynamics is used, as in (4–5), to update the positions of the filament beads experiencing the above forces, following the Langevin Equation (6):

$\mathbf{F}_i^{\text{spring}} + \mathbf{F}_i^{\text{bend}} + \mathbf{F}_i^{\text{thermal}} + \mathbf{F}_i^{\text{crslnk}} + \mathbf{F}_i^{\text{myo}} = \zeta_b d\mathbf{r}_i/dt$, where $\mathbf{r}_i$ is the position of the i th bead, ζ_b is an effective drag coefficient. $\mathbf{F}_i^{\text{spring}}$, $\mathbf{F}_i^{\text{bend}}$, $\mathbf{F}_i^{\text{thermal}}$, $\mathbf{F}_i^{\text{crslnk}}$, and $\mathbf{F}_i^{\text{myo}}$ are forces due to: springs, bending, thermal fluctuations, cross-linking between actin filaments and node pulling. A similar equation governs the movement of nodes along the membrane, with a larger drag coefficient representing cortical friction. The model is developed using Java and Open Source Physics. We tested that the code reproduces single filament persistence length, relaxation dynamics, and energy equipartition.

RESULTS

Our numerical 3D model reproduces contractile ring assembly within 10 minutes, consistent with experiments (2, 3). In the simulations, filaments grow along random directions, get pulled by myosin in other nodes and align with other filaments through cross-linking. Variations in r_c are correlated with morphological transitions and changes in the distribution of forces on individual actin filament springs and on cortical nodes, leading to rings, transient meshworks, or clumps (Fig. 1B). These configurations correspond to morphologies observed in wild type and mutant cells with varied concentration of actin filament cross-linkers (2, 7).

Forces on individual nodes are intermittent and can be discretized into a series of two states, below or above 0.5 pN , see Fig. 2A. The distribution of forces on individual nodes changes over time, as illustrated in Fig. 2B: in the first minute (early assembly) most nodes bear forces below 1 pN , with only a small fraction of nodes reaching 10 pN ; after 10 min (late ring assembly), forces are more homogenous, with values between 1 and 6 pN . Varying r_c , clumps or meshworks form and the average microscopic forces on individual nodes vary between 1 – 4 pN (Fig. 2C).

Contractile forces generate tension along filaments (color map in Fig. 3A) and on nodes (Fig. 3B). For r_c values from 0.09 to $0.14 \mu\text{m}$ that lead to contractile rings, forces initially pull nodes towards the middle of the cell (Fig. 3B); as the band condenses, forces on nodes become constricting. This can lead to membrane deformation (not included in the simulations). We predict tension along actin filaments is distributed approximately uniformly through the cross-linked network with transient spots of high extensile forces, usually when an unbundled filament is captured and pulled (Fig. 3A).

With $r_c=0.16\text{ }\mu\text{m}$, a meshwork can form and the microscopic forces on the cortical nodes have anisotropic directions (Fig. 4A). With $r_c<0.09\text{ }\mu\text{m}$, clumps of nodes form, with anisotropic forces on nodes (Fig. 4B).

CONCLUSIONS

This study focuses on the relation between microscopic tensions on cortical nodes and actin filaments and macroscopic morphological transitions of an actin network into rings, clumps or transient meshworks. Our numerical results show a specific pattern of force evolution during contractile ring assembly: in early stages of ring formation, forces on cortical nodes help narrowing the broad band, whereas at later stages, these forces re-orient towards the cell interior, as shown experimentally in (8,9), and can subsequently aid in ring constriction. This pattern of force evolution is modified in mutant cells, leading to non-functional aggregates.

Acknowledgments

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Figure 1. Computational 3D domain and main morphological transitions of the actin network (A) Cartoon of 3D model (nodes in red; actin filaments in green). (B) Side view and cross sections of initial actomyosin configuration, ring, clumps, meshwork for different r_c (medium, low, and high, respectively).

Figure 2. Microscopic forces on individual nodes during cytokinetic ring assembly
 (A) Forces on nodes are intermittent. (B) Distribution of forces on nodes varies during ring assembly. (C) Average force on nodes varies with r_c (low, circles; medium, asterisks; high, crosses).

Figure 3. Extensile and contractile forces on actin filaments and force vectors acting on cortical nodes during ring assembly

(A) Maximum force on the actin units decreases during ring assembly. Scale: pN. (B) Vectors of forces acting on nodes face toward the cell center at late assembly (10 min). Average arrow length is 3 pN.

Figure 4. Extensile and contractile forces on actin filaments and force vectors on nodes in meshworks and clumps

(A) Forces on the actin units and vectors of forces acting on cortical nodes for meshworks and (B) clumps.