Model of Yeast Actin Cable Distribution and Dynamics

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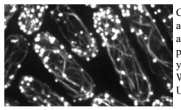
Abstract

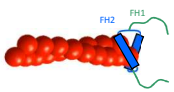
The growth of fission yeast relies on the polymerization of actin filaments at cell tips. These filaments are nucleated by formin proteins that localize at tip cortical sites. These actin filaments bundle to form actin cables that span the cell and guide the movement of vesicles toward the cell tips. Since actin cables are structures whose dynamics can be monitored by fluorescence microscopy, and since yeast is a tractable genetic system, comparison of the results of theoretical models of actin cables to experiment could enable quantitative tests of the mechanisms of actin polymerization in cells. We used computer simulations to study the spatial and dynamical properties of actin cables. We simulated actin individual filaments as semiflexible polymers in 3D, composed of beads connected with springs. Formin polymerization was simulated as filament growth out of cortical sites located at cell tips. Actin filament severing by cofilin was simulated as filament turnover. We added attractive interactions between beads to simulate filament bundling by actin cross-linkers such as fimbrin. Comparison of the results of the model to prior experiments suggests that filament severing, nucleation and crosslinking are sufficient to describe the many features of actin cables. We found bundled and unbundled phases as cross-linking strength was varied and propose experiments to test the model predictions.

Introduction

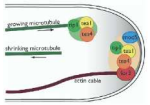
- Formin For3p proteins on the cortical sites of the cell tip nucleates actin monomers, promoting actin polymerization to form actin filaments.
- Actin cables are bundles of filaments aligned along the axis of fission yeast.



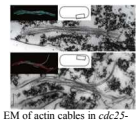
CHD-GFP labeled actin displaying actin cables and patches in fission yeast . Jian-Qiu Wu (Ohio State Univ.)



For3p associate with barbed ends and promote actin polymerization.

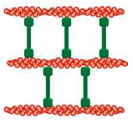


Actin cables: bundles of actin filaments nucleated by formin For3p. S. Martin and F. Chang, Dev. Cell (2006)

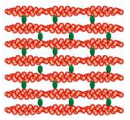


EM of actin cables in *cdc25-22*. T Kamasaki et al., Nature Cell Biol. (2005)

- Two actin filament cross-linkers in fission yeast, α-actinin (ain1p) and fimbrin (fim1p), which can bundle actin filaments into a parallel or antiparallel array. (C Skau et al. JBC(2011).)
- Motor protein myosin V, carrying secretory vesicles or anchoring on organelles, walks along actin filament towards the barbed end.

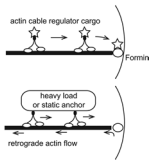


Actin filaments and α-actinin



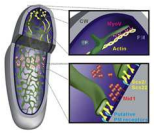
Actin filaments and fimbrin

Molecular Biology of the Cell by Alberts et al.

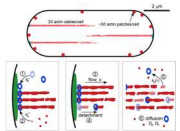


Possible mechanism of actin regulation by Myosin V. L Presti, F Chang and S Martin, MBoC (2012).

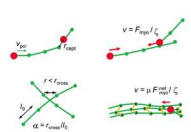
- The endoplasmic reticulum (ER) is attached to the plasma membrane, anchored by myosin V.
- For3p-mediated actin cable assembly, and bundling of actin filaments have been previously modeled in fission yeast.
- We aim to build a 3D model of actin cables in yeast cell based on these models.



Model for cortical ER arrangement. D Zhang, A Vjestica, S Oliferenko, Current Biology (2012)



Model of For3p-mediated actin cable assembly. H Wang and D Vavylonis, Plos One (2008)

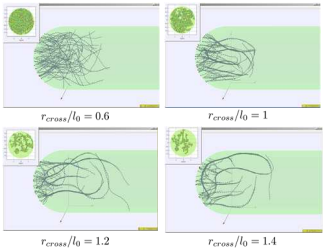


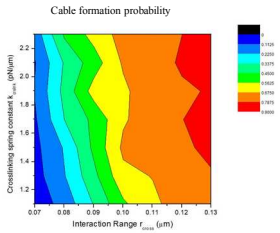
Interactions between actin filaments during contractile ring formation. D Laporte, N Ojkic, D Vavylonis, JQ Wu, MBoC (2012)

RESULTS

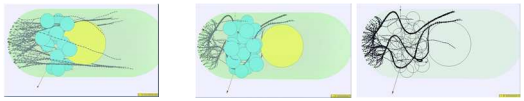
Cross-linking bundles actin filaments into cables

- Higher crosslinking simulated by longer interaction range leads to thicker and fewer cable formations.
- Cross-section at 2μm plane, indicates distribution of filaments over time (inset).
- Cable formation is predominantly determined by interaction range with small effect due to the change in cross-linking spring constant (far right).

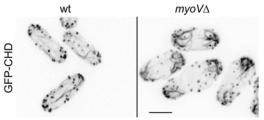




Myosin V pulling stretches actin cables



With myosin walking | Without myosin walking

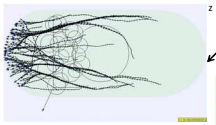


L Presti, F Chang and S Martin, MBoC(2012)

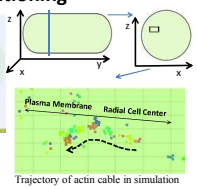
- In simulations myosin V straightens and stiffens the actin cables relative to simulations without myosin V.
- Compares favorably to observations of actin cables in wild-type and myoVΔ, where cells without myosin V exhibit straightening cable defect.

ER attachment to PM contributes to actin cable positioning

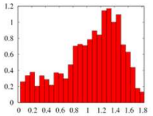
- Trajectory shows that actin cables move towards the cell membrane on average.
- Myosin proteins anchor on ER thus influencing the distribution of actin cables.
- Analysis of experimental images shows that cables localize near cell wall (far right).



Distribution of actin cables



Trajectory of actin cable in simulation



Radial actin cable distribution. Experimental data extracted using Jfilament. Cables marked with CHD-GFP (E Yusuf, I-J Lee, J-Q Wu, D Vavylonis)

Three-Dimensional Model of Actin Cables

1. Model of Single Actin Filament

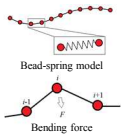
- Bead-spring model is used to simulate a single actin filament in 3D. Beads sitting on the backbone of the filament connected by springs represent the shape of the actin filament.
- Spring, bending and thermal forces together influence the dynamics of the actin filament.
- Equilibrium and dynamic properties such as persistence length, mode relaxation time, equipartition theorem have been verified.
- Langevin dynamics:

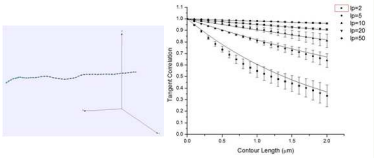
$$\vec{F}_i^{spring} = -\frac{\partial E^{spring}}{\partial \vec{r}_i} = -\frac{k}{2}\sum_{j=1}^{N-1}\frac{\partial(|\vec{r}_{j+1}-\vec{r}_j|-l_0)^2}{\partial \vec{r}_i}$$

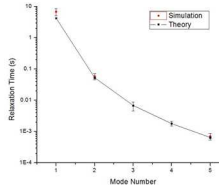
$$\vec{F}_i^{bend} = -\frac{\partial E^{bend}}{\partial \vec{r}_i} = -\frac{\kappa}{l_0}\sum_{j=2}^{N-1}\frac{\partial(\vec{t}_j\cdot\vec{t}_{j-1})}{\partial \vec{r}_i}$$

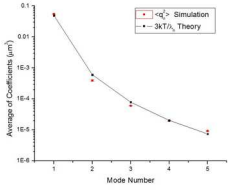
$$\langle \vec{F}_i^{thermal}\vec{F}_i^{thermal}\rangle_{\alpha,\beta} = \frac{2k_BT\zeta_b}{\Delta t} = \hat{I}_{\alpha,\beta}$$

$$\vec{F}_i^{spring} + \vec{F}_i^{bend} + \vec{F}_i^{thermal} = \zeta_b\frac{d\vec{r}_i}{dt}, \quad i = 1, 2, ..N$$









Three-Dimensional Model of Actin Cables

2. Model of Actin Cables inside a Fission Yeast Cell

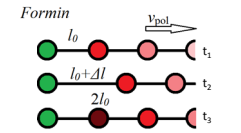
- Fission yeast cell boundary is simulated as a fixed cylinder capped by hemispheres at both ends.
- Formin proteins polymerize actin filaments at barbed end on the cortical sites of the tips.
- Short range attraction simulates the cross-linking proteins binding actin filaments to form cables or networks.

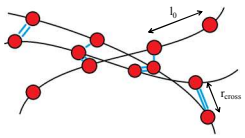
$$\vec{F}_i^{crslnk} = -\frac{k_{crslnk}}{2}\sum_j\frac{\partial(|\vec{r}_i-\vec{r}_j|-r_0)^2}{\partial \vec{r}_i}$$

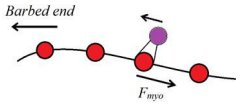
- Myosin V binding simulated as a transient constant force in the tangential direction towards the barbed end.

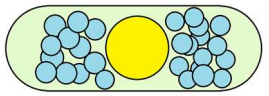
$$\vec{F}_i^{myosin} = -F_{myosin}\cdot\vec{t}_{i-1}$$

- Severing by cofilin simulated as filament turnover.
- Nucleus is simulated as an immobile sphere at the center of the cell.
- Vacuoles are approximated as immobile spheres randomly distributed inside the cell, limiting the volumes accessible to filaments.
- The effect of tethering of ER to cell wall is simulated as an outward radial force on myosin.

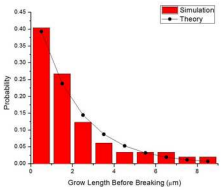


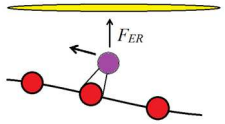






Parameter	Description	Value
N	Number of filaments	100
D_{yeast}	Yeast diameter	$3.6\mu m$
L_{yeast}	Yeast length	$10\mu m$
t_{sever}	Characteristic severing time	$20s$
k_{crslnk}	Crosslinking spring constant	$2pN/\mu m$
r_0	Gap distance between filaments	$0.03\mu m$
r_{cross}	Interaction range of crosslinking	$0.1\mu m$
v_{pol}	Polymerization rate at tip	$0.1\mu m/s$
l_0	Equilibrium length of spring	$0.1\mu m$
T	Temperature	$300K$
k	Spring constant	$2pN/\mu m$
l_p	Persistence length	$10\mu m$
η	Viscosity	$0.301Pa\cdot s$
dt	Simulation time step	$0.0002s$
F_{myo}	Myosin strike force	$5pN$





Summary & Conclusions

- Simulated 3D distribution and dynamics of actin cables in fission yeast.
- Model included formin polymerization, cross-linking, turnover, myosin V pulling.
- Cross-linking (possibly via fimbrin) generates bundles of actin filaments that resemble actin cables even when filament polymerization occurs randomly along cell tip.
- Longer persistence length leads to straighter and less tangled cables.
- Simulated myosin V walking stiffens the actin cables and prevents cables from tangling, in agreement with prior experiments.
- Radial pulling, simulating ER attachment influences the radial distribution of actin cables.

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