Model of Yeast Actin Cable Distribution and Dynamics

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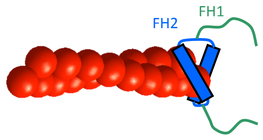
Abstract

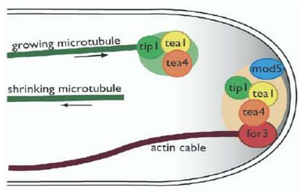
The growth of fission yeast relies on the polymerization of actin filaments at cell tips nucleated by formin For3p that localizes at tip cortical sites. These actin filaments bundle to form actin cables that span the cell and guide the movement of vesicles toward the cell tips. Since actin cables are 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 individual actin 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

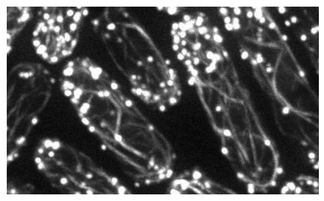
- Formin For3p proteins on the cortical sites of the cell tip nucleate actin filaments that form bundles aligned along the axis of fission yeast.



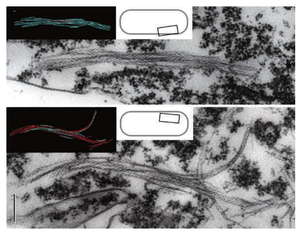
For3p nucleates actin filaments and associates processively with barbed ends



S. Martin and F. Chang, Dev. Cell (2006)

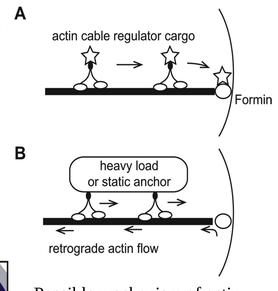


CHD-GFP labeled actin displaying actin cables and patches in fission yeast . Jian-Qiu Wu (OSU)



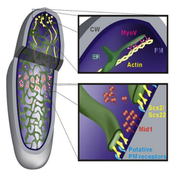
EM of actin cables in *cdc25-22*. Kamasaki et al., Nature Cell Biol. (2005)

- Two actin filament cross-linkers in fission yeast, α-actinin (Ain1p) and fimbrin (Fim1p), can bundle actin filaments into parallel or antiparallel orientations. (C. Skau et al. JBC (2011), Laporte et al., MBoC (2012))

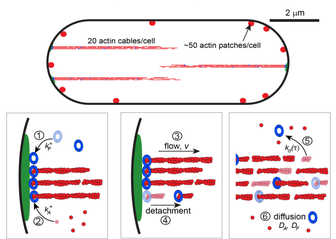


Possible mechanism of actin regulation by Myosin V. L Lo Presti, F Chang and S Martin, MBoC (2012).

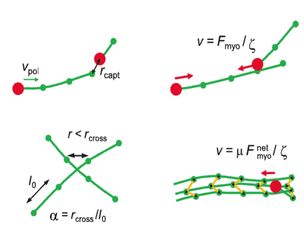
- Motor protein myosin V, carrying secretory vesicles or anchoring on organelles, walks along actin filament towards the barbed end.
- The ER is attached to the plasma membrane and to cables by myosin V. D Zhang, A Vjestica, S Oliferenko, Curr Biol (2012).



- For3p forms clusters at cell tips. Martin and Chang, Dev. Cell (2006), J. Dodgson et al. Nature Comm. (2013).
- For3p-mediated actin cable assembly, and bundling of actin filaments have been previously modeled in fission yeast.



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

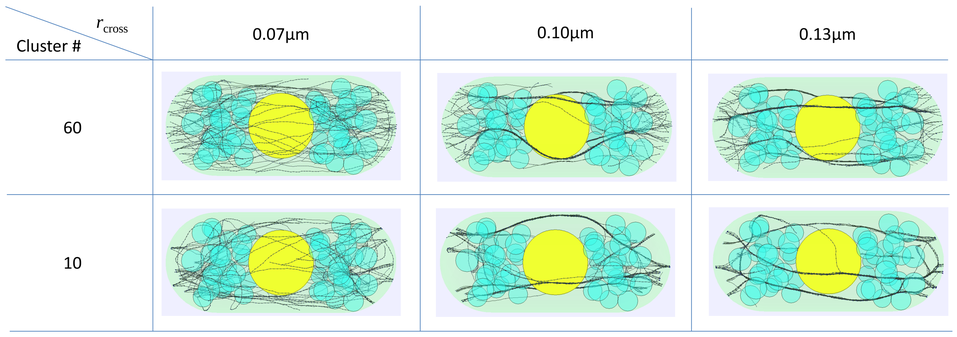


Interactions between actin filaments during contractile ring formation. D Laporte, N Ojkic, D Vavylonis, JQ Wu, MBoC (2012)

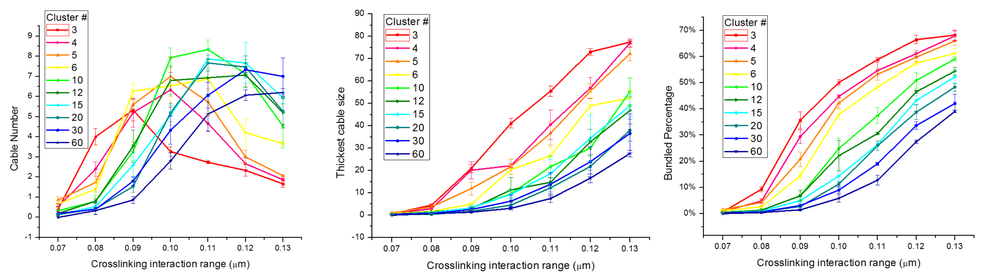
RESULTS

Formin clustering at cell tips and cross-linking affect cable formation

- Higher crosslinking (simulated by longer interaction range r_{cross}) leads to thicker and fewer bundles
- Denser formin clusters (smaller # clusters under fixed total filament number) enhances actin cable formation.

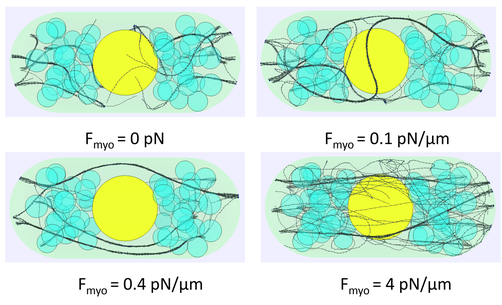


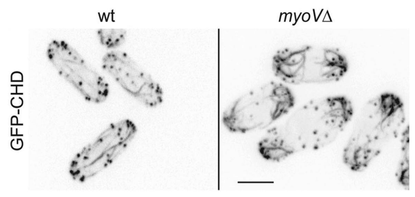
- The effect of increase of crosslinking involves two stages: 1. Individual actin filaments bundle into cables; 2. Small actin cables merge and bundle together to form thicker but fewer cables



Myosin V pulling stretches actin cables

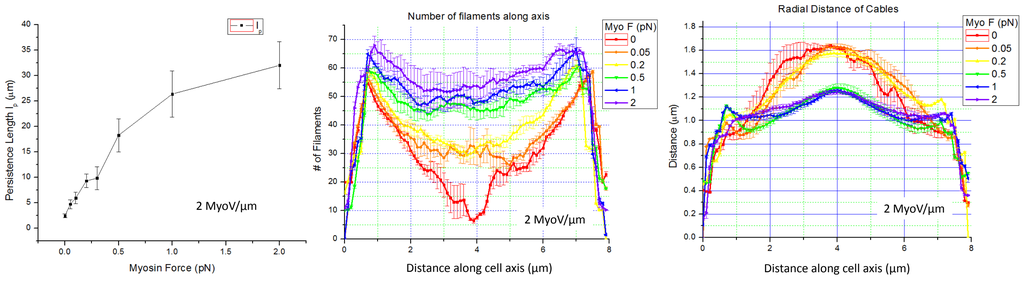
- In simulations myosin V straightens and stiffens the actin cables relative to simulations without myosin V.
- Simulated system compares favorably to observations of actin cables in wild-type and myoVΔ, where cells without myosin V exhibit straightening cable defect.
- Large myosin force competes with crosslinking force and leads to fewer bundled actin filaments (figure panel bottom right and figure on right).





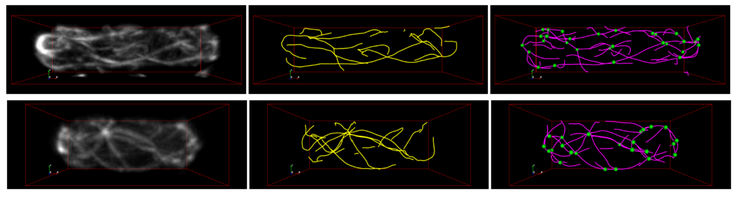
L Lo Presti, F Chang and S Martin, MBoC(2012)

- Without myosin V pulling, cables get tangled up near the cell tips



Ongoing work: compare to automated 3D segmentation results

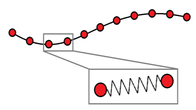
Multiple stretching open active contours (SOAX) (Medical Image analysis, submitted)



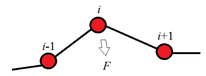
Ting Xu,, I-Ju Lee, E. Yusuf, D. Vavylonis, J.-Q. Wu, X. Huang

Three-Dimensional Model of Actin Cables

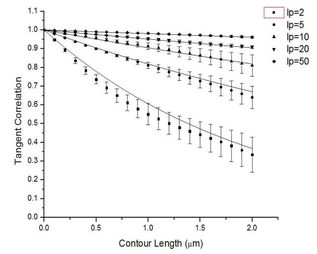
- Bead-spring model is used to simulate a single actin filament in 3D.
- Spring, bending and thermal forces together influence the dynamics of the actin filament.
- Equilibrium and dynamic properties such as persistence length, mode relaxation time, equipartition theorem have been verified.

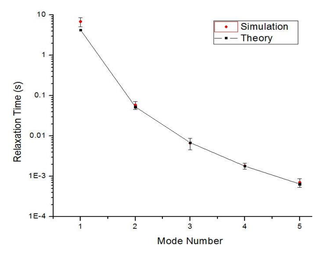


Bead-spring model



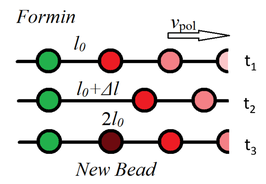
Bending force

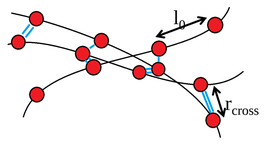




- Fission yeast cell boundary is simulated as a fixed cylinder capped by hemispheres at both ends.
- Formin proteins polymerize actin filaments at barbed end on the cortical sites of the tips.
- Short range attraction simulates the cross-linking proteins binding actin filaments to form cables or networks.

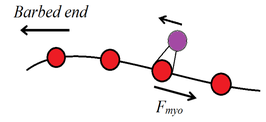
$$\vec{F}_i^{crslnk} = -\frac{k_{crslnk}}{2}\sum_j \frac{\partial(|\vec{r}_i - \vec{r}_j| - r_0)^2}{\partial \vec{r}_i}$$



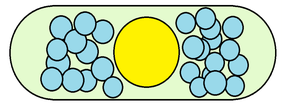


- Myosin V binding simulated as a transient constant force in the tangential direction towards the barbed end.

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

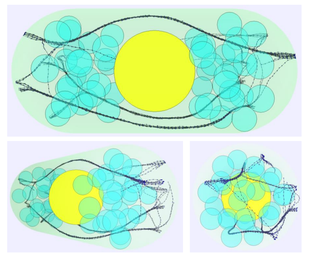


- Severing by cofilin simulated as filament turnover.
- Nucleus is simulated as an immobile sphere at the center of the cell.
- Vacuoles are approximated as immobile spheres randomly distributed inside the cell, limiting the volumes accessible to filaments.



Parameter	Description	Value
N	Number of formins per tip	60^*
D_{yeast}	Yeast diameter	$3.6\mu m$
L_{yeast}	Yeast length	$8\mu m$
l_{sever}	Characteristic severing length	$3\mu m$
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}	Average myosin force	$0.2pN^*$

*: System simulated under various parameter values.



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.
- Myosin pulling force affects the cable distribution and radial distance at the cell center.

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