Organization of actin cables in budding yeast: a computational model

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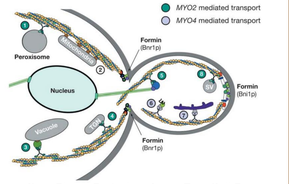
Abstract

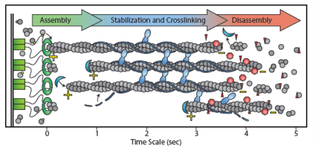
Budding yeast actin cables function as tracks for delivery of cellular organelles to the growing bud during polarized cell growth and aid in spindle orientation during mitosis. For proper cellular function, actin cables must form polarized bundles that grow from the bud to the mother, spanning the whole cell. Overexpression and disruption of Bni1 leads to changes of actin cables structures. Deletion of Smy1p causes longer cables with occasional bends and turns. Here, we develop a 3D computational model of actin cable dynamics to study the interplay between molecular mechanisms and large scale organization of actin cables in budding yeast. This model extends our prior study of actin cables in fission yeast (Tang et al. MBoC 2014). In this model, formins Bni1 and Bnr1 promote actin filament barbed end polymerization. The actin filaments are simulated as semiflexible polymers composed of beads connected by springs. Formin turnover is incorporated as filament detachment and recruitment at cortical sites. Cross-linking by fimbrin Sac6 is simulated as a short-range attractive interaction. Pulling by class V myosin and class II myosin are simulated as tangential forces on filament beads. Cofilin-induced severing is implemented as breaking and disassembly of filament segments. We show that with these mechanisms, the model can generate the actin cable structures similar to those observed in experiments. Varying the model parameters, such as crosslinker density, severing probability, and formin turnover, reproduces actin cable structure observed in cell mutants. We also show that Bnr1p locations affect Bnr1p nucleated cable polarity. These simulations illustrate how biophysical and biochemical properties combine to establish different types of cytoskeletal organization at the cellular scale.

Introduction

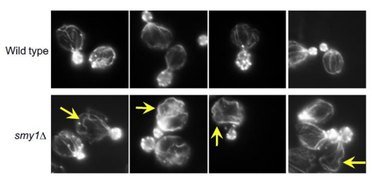
- In budding yeast, formins Bni1p localizing at the bud tip and Bnr1p near the bud neck nucleate actin filaments that form bundles spanning the whole cell.
- Actin filaments are bundled by crosslinking proteins Fimbrin Sac6p.
- Actin filaments are stabilized by tropomyosin tpm1p that binds to the side of it and aged segments are severed by cofilin Cof1p.
- Motor protein myosin V, myo2p in budding yeast, carrying secretory vesicles, (mitochondia?) or Smy1p, walks along actin filament towards the barbed end. Smy1p is continually delivered to Bnr1p sites and serves as a negative regulator of actin polyemrization.
- Type II myosin Myo1p in budding yeast, localizing near the bud neck, generates pulling force on actin cables.



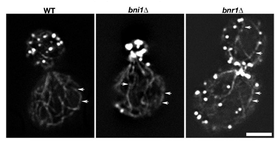
Pruyne, Gao, Bi and Bretscher, Annu. Rev. Cell Dev. Biol. (2004)



Goode's Lab poster. (replace with published results.)

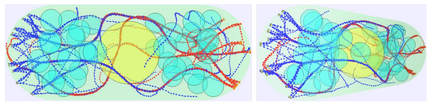


Chesarone-Cataldo, Gue´rin et al. Goode Dev. Cell (2011)

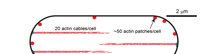


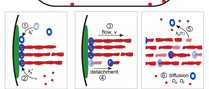
SM Buttery, S Yoshida and D Pellman, MBoC (2007)

- 3D computation model of actin cables and cytokinetic ring in fission yeast: a model that includes formin, crosslinkers α-actinin, and fimbrin, myosin V, and a turnover mechanism.

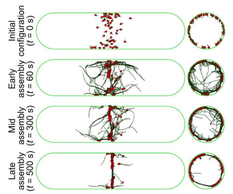


Tang, Laporte, Vavylonis, MBoC (2014)





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

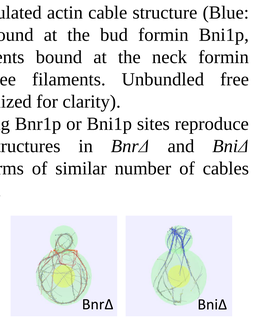


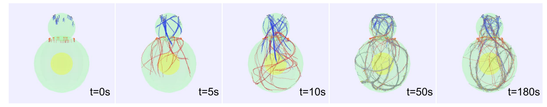
Bidone, Tang, Vavylonis, Biophy. J. (2014)

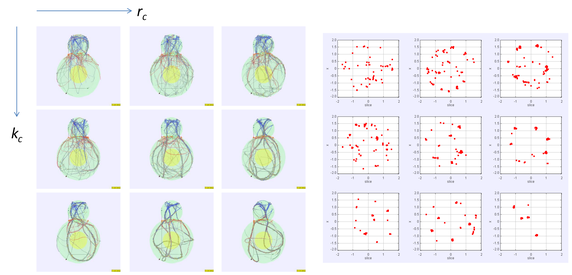
RESULTS

Model resembles actin cable structure in budding yeast

- Time lapse of simulated actin cable structure (Blue: actin filaments bound at the bud formin Bni1p, Red: actin filaments bound at the neck formin Bnr1p, Grey: free filaments. Unbundled free filament not visualized for clarity).
- Simulations lacking Bnr1p or Bni1p sites reproduce similar cable structures in *BnrΔ* and *BniΔ* experiments in terms of similar number of cables and thinner cables.





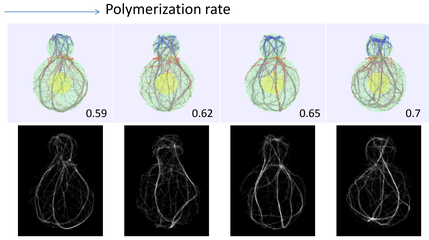


Crosslinking affects cable formation

- Increasing crosslinking parameters r_c and k_c helps bundling actin filaments.
- As cross-linking parameters increase, cable number first increases because bundles start to form, but then decreases as thin cables bundle to form thick cables.

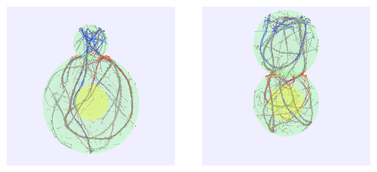
Increasing polymerization rates to simulate *Smy1Δ*

- Increasing polymerization rate generates thicker and longer cables.
- There are occasional turns and bends in such cables.



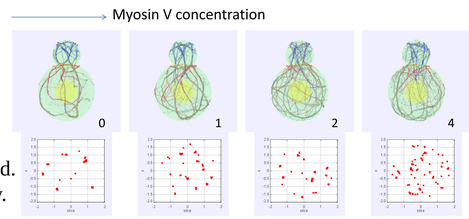
Geometry change and its effect on actin cables

- We explore further the geometry variations by changing the sphere radius for bud and mother.
- Big bud -> clearer cable features
  Small bud -> tangled actin filament network
  Big mother -> sparse, straighter cables
  Small mother -> more complex, convolved cable network.



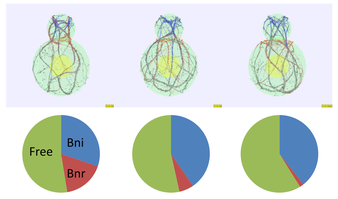
Myosin V transportation affects cable properties

- In *Myo2Δ* cells, cables are thick and curved
- Increasing myo2p concentration induces stronger walking force, unbundles thick cables, generating thin, straight cables.

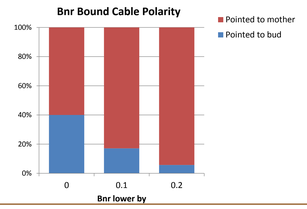


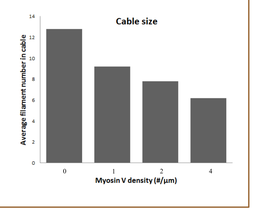
Bnr1p location affects cable polarity

- Bnr1p formin location affects the cable composition in the bud.
- Lowering Bnr1p formin sites helps maintaining cable polarity.



Filaments in the bud



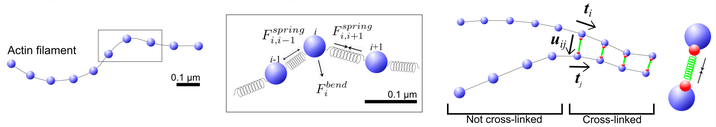


Model

- Coarse-grained bead-spring model is used to simulate a single actin filament in 3D.

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

- Spring, bending and thermal forces together influence the dynamics of the actin filament.

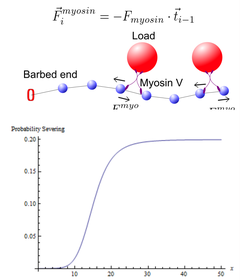


- Crosslinking is simulated by short range attraction with angle preference:

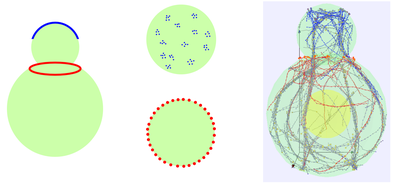
$$\mathbf{F}_i^{crslnk} = -\frac{k_{crslnk}}{2}\sum_j \frac{\partial(|\mathbf{r}_i-\mathbf{r}_j|-r_0)^2}{\partial \mathbf{r}_i}, \text{ for } |\mathbf{r}_i-\mathbf{r}_j| \le r_c \text{ and } \mathbf{u}_{ij}\cdot\mathbf{t}_i < 0.5 \text{ and } \mathbf{u}_{ij}\cdot\mathbf{t}_j < 0.5$$

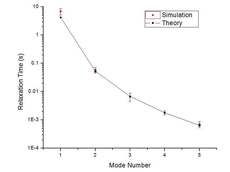
- Bni1p at the tip and Bnr1p at the neck polymerizes actin filaments at rates of 0.29 and 0.59 μm/s, respectively. It is simulated as elongation of the equilibrium length of the first segment .
- Myosin V binding simulated as a transient tangential force towards the barbed end.
- Severing rates by cofilin are different for ATP-actin, ADPPi-actin and ADP-actin. This aging mechanism is simulated as a hill function. Cof1p binds and breaks the filament into two segments at the severing site.
- Myosin II contractility is simulated as a tangential force towards the barbed end similar to the Myosin V force and a outward pulling force.
- The shape for budding yeast:
  mother sphere radius 2 μm centered at (0, 0, 0);
  bud sphere radius 1 μm centered at (0, 0, 2.6 μm);
  nucleus sphere radius 0.8 μm centered at (0, 0, 0);
  Bnr1p formins lowered from the neck for 0.1 μm.

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



- Equilibrium and dynamic properties such as persistence length, mode relaxation time, equipartition theorem have been verified.





Parameter	Description	Value	Parameter	Description	Value
N_{bud}	Number of bud formins Bni	55	k_{crslnk}	Cross-linking spring coefficient	5, 2→10pN/μm
$N_{cluster}$	Number of clusters of Bni	10, 5->55	r_0	Cross-linking gap distance	0.02μm
N_{neck}	Number of neck formins Bnr	35	r_{crslnk}	Cross-linking interaction range	0.1, 0.07→0.12μm
R_{mother}	Radius of the mother sphere	2, 1.5->2.3μm	F_{myoV}	Myosin V walking force	0.5pN
R_{bud}	Radius of the bud sphere	1, 0.8->1.5μm	$F_{myoII,\ tan}$	Myosin II pulling force, tangential	0.5pN
$R_{nucleus}$	Radius of the nucleus sphere	0.8μm	$F_{myoII,\ out}$	Myosin II pulling force, outward	0.5pN
z_{neck}	Postion of the neck	1.9μm	[MyoV]	Myosin V linear density	1, 0->10#/μm
$v_{pol,bni}$	Polym. rate for Bni actin filament	0.29, 0.25->0.4μm/s	[MyoII]	Myosin II binding probability	1, 0->10#/μm
$v_{pol,bnr}$	Polym. rate for Bnr actin filament	0.59, 0.4->0.7μm/s	τ_{myo}	Myosin V binding lifetime	5s
$v_{pol,free}$	Polym. rate for free actin filament	0μm/s	τ_{age}	Actin monomer aging time	15s
l_p	Persistence length of the F-actin	15, 10->20μm	$r_{actin,\ max}$	Maximum severing probability	0.2
l_0	Filament model segment length	0.1μm			

Summary & Conclusions

- Simulated 3D distribution and dynamics of actin cables in budding yeast, including formin polymerization, cross-linking, turnover, myosin V and myosin II pulling.
- Cross-linking generates bundles of actin filaments that resemble actin cables
- Smy1Δ mutant cells are simulated by increasing polymerization rate of actin filaments, which generates longer, thicker cables with occasional bends and turns.
- Simulated myosin V walking stiffens the actin cables and unbundles cables.
- Bnr1p sites affect bnr bound cable polarity.

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