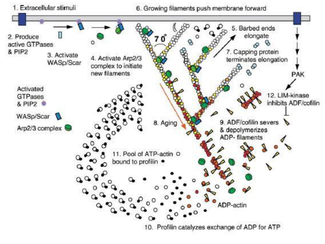
Model of Capping Protein and Arp2/3 Complex Turnover in the Lamellipodium Based on Single Molecule Statistics

Laura McMillen, M.B. Smith, D. Vavylonis Physics, Lehigh University, Bethlehem, PA

Capping protein (CP) and Arp2/3 protein complex regulate actin polymerization near the leading edge of motile cells. They assemble near the edge of the lamellipodium, undergo retrograde flow, and dissociate into the cytoplasm as single subunits or as part of actin oligomers. To better understand this cycle, we modeled the kinetics of CP and Arp2/3 complex in the lamellipodium using data from prior single molecule microscopy experiments [Miyoshi et al. JCB, 2006, 175:948]. In these experiments speckle appearance and disappearance events corresponded to assembly and dissociation from the F-actin network. We used the measured dissociation rates of Arp2/3 complex and CP (0.048 s^{-1} and 0.58 s^{-1}, respectively) in a Monte Carlo simulation that includes particles in association with F-actin and diffuse in the cytoplasm. We explored the effect of slowly diffusing cytoplasmic pool to account for a big fraction of CP with diffusion coefficients as slow as 0.5 $\mu m^2/s$ measured by single molecule tracking [Smith et al. Biophys. J., 2011,101:1799]. These slowly diffusing species could represent severed actin filament fragments. We show that such slow diffusion coefficients are consistent with prior FRAP experiments by Kapustina et al. [Cytoskeleton, 2010, 67:525] who fitted their data using larger diffusion coefficients. We also show that the single molecule data are consistent with FRAP experiments by Lai et al. [EMBO J., 2008, 28:986] who found that the Arp2/3 complex recovers more quickly at the front of the lamellipodium as compared to the back. We discuss the implication of disassembly with actin oligomers and suggest experiments to distinguish among mechanisms that influence long range transport.

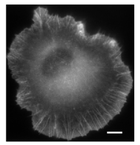
1. Introduction

- The lamellipodia contains a dense, dynamic actin network [right]
- At the back of the network F-actin breaks up into Oligomers and Monomers to be recycled for polymerization to the network [right]



Pollard and Borisy. *Cell* 112, 4 (2003).

A



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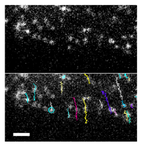

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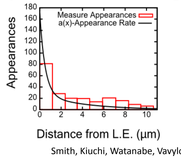

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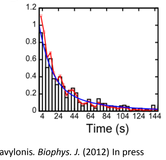
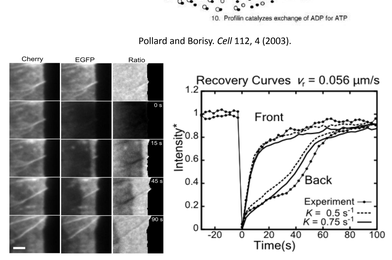


Smith, Kiuchi, Watanabe, Vavylonis. *Biophys. J.* (2012) In press

- Single Molecule microscopy (speckle) data → appearance and lifetime distributions [above] (XTC cells)

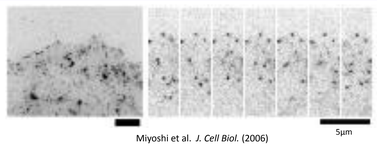
Our goal: adapt model that works for actin to CP and Arp2/3 complex and compare to experiments



Smith, Kiuchi, Watanabe, Vavylonis. *Biophys. J.* (2012) In press

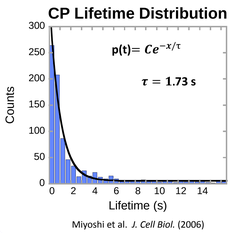
- Smith et al. find that in FRAP of EGFP-actin recovery away from the leading edge lags recovery at the front [above]
- Fitting this with a model, they find that model involving oligomers as a diffuse species fits experiment very well [above]

2. Capping Protein Dynamics at the Leading Edge

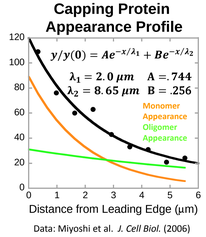


Miyoshi et al. *J. Cell Biol.* (2006)

- CP exhibits fast turnover when imaged at the single molecule level in XTC cells
- Broad distribution of appearances away from the leading edge
- Appearance rate can be split into two exponentials (monomers near the leading edge + oligomers further away from the leading edge)

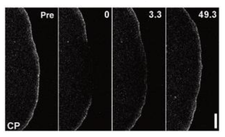


Miyoshi et al. *J. Cell Biol.* (2006)



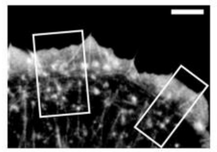
Data: Miyoshi et al. *J. Cell Biol.* (2006)

Other studies: narrower CP distribution (B16-F1 Melanoma Cells)

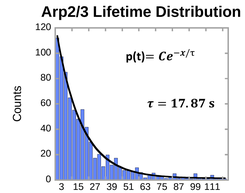


Lai et al. *EMBO J.* (2008)

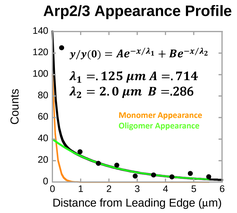
3. Arp2/3 Complex Dynamics at the Leading Edge



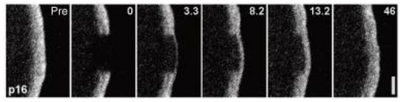
Miyoshi et al. *J. Cell Biol.* (2006), XTC cells



Data: Miyoshi et al. *J. Cell Biol* (2006)



Data: Miyoshi et al. *J. Cell Biol.* (2006)

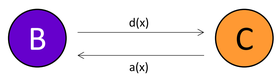


Lai et al. *EMBO J.* (2008), B16-F1 Melanoma cells
FRAP experiment shows recover profile

- Fit to experimental data suggests a spike of appearance rate for monomers close to the leading edge

4. Model

- This work is an extension of Smith, Kiuchi, Watanabe, Vavylonis, *Biophys J.* (2012) In press
- Actin network is treated as a field with regions of polymerization and depolymerization
- Cytoplasmic protein diffuses freely through the network
- Protein that binds to the network moves away from the leading edge with retrograde flow v_r



$$a(x) = G_\infty K\left(A_1 e^{-x/\lambda_1} + A_2 e^{-x/\lambda_2}\right)$$

Appearance rate *a(x)* and lifetime distribution *p(t)* determine the Bound protein profile

$$B(x) = \frac{1}{v_r}\int_0^x \int_{\frac{x-x'}{v_r}}^{\infty} p(t)a(x')dtdx'$$

$$0 = v_r\frac{\partial B}{\partial x} - d(x) + a(x)$$

$$0 = D\frac{\partial^2 C}{\partial x^2} + d(x) - a(x)$$

From the Bound protein profile we can find the Cytoplasmic protein distribution

$$C(x) = C_\infty - \frac{v_r}{D}\int_x^\infty B(x)dx$$

C_∞ is the concentration of labeled cytoplasmic protein away from leading edge

K Strength of polymerization rate. Adjusts bound to cytoplasmic protein ratio

The steady state for cytoplasmic protein determines the binding rate in a 2D Monte Carlo simulation:

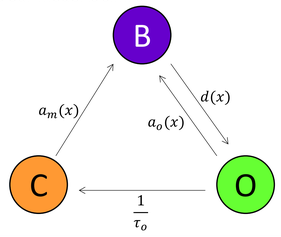
$$r_C(x) = \frac{a(x)}{C(x)}$$

For all simulations a value of .03 μm/s was used for v_r [Ryan et al. *Biophys J.*(2012)]

Presented above is the model for free cytoplasmic proteins as only diffuse species. CP or Arp2/3 complex can also be bound to disassembling actin oligomers, where our model is similar:

Appearance rate for the Oligomer Model is broken up into two separate appearance rates

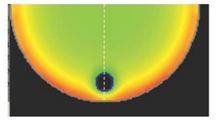
$$a(x) = a_o(x) + a_m(x)$$



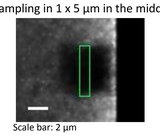
5. Simulation of Capping Protein and Arp2/3 Complex Turnover

Capping Protein

Simulated FRAP from Kapustina et. al. *Cytoskeleton* (2010)



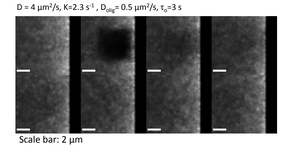
Simulated FRAP in our model. Bleached region: 5 x 5 μm, 0.5 μm from leading edge. Sampling in 1 x 5 μm in the middle



Scale bar: 2 μm

Simulated montage of CP recovery. Time per frame: 2 s
K is chosen from analysis of speckle data

D = 4 $\mu m^2/s$, K=2.3 s^{-1} , D_{olig}= 0.5 $\mu m^2/s$, τ_o=3 s



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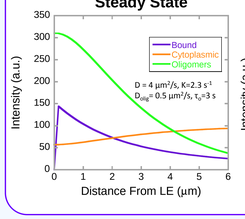
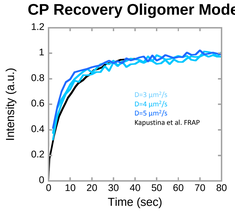
CP Oligomer Model Steady State



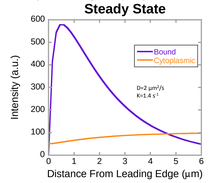
CP Recovery Oligomer Model



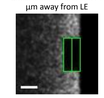
- Miyoshi et al. measure a very short lifetime for bound CP, τ = 1.73 s
- Smith et al. (Biophys J 2011) see a large pool of slowing diffusing CP with a diffusion constant 0.5 $\mu m^2/s$
- Kapustina et al. (2010) model best fit: k_{off} = .1 s^{-1} which corresponds to a bound CP lifetime of τ = 10 s. Best fit for diffusion coefficient D = 5-10 $\mu m^2/s$
- Our model fits the Kapustina et al. experimental data but with a short lifetime as in Miyoshi et al. (τ = .962 s), a lifetime of τ_o = 20 s for oligomers, a range of diffusion coefficients for free protein between D = 3-5 $\mu m^2/s$, and a large pool of oligomers with a diffusion coefficient of D_{olig} = 0.5 $\mu m^2/s$
- Model without oligomers also fits well with data except that a small cytoplasmic diffusion coefficient is needed (D ~ 1 $\mu m^2/s$)

Arp2/3 Complex

Arp2/3 Single Cytoplasmic Pool Steady State

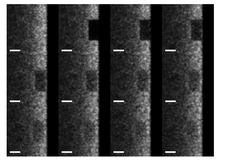


Front: 1 μm wide by 4 μm high at LE
Back: Same dimensions 1 μm away from LE



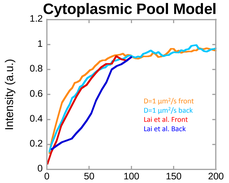
Scale bar: 2 μm

Arp2/3 Monomer Model Simulated Recovery
Bleach area: 2 x 4 μm. D=2 $\mu m^2/s$, K=1.4 s^{-1}

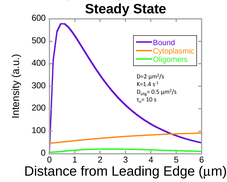


Scale bar: 2 μm, 3 s/frame

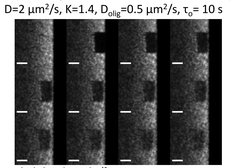
Arp2/3 Recovery Single Cytoplasmic Pool Model



Arp2/3 Oligomer Model Steady State

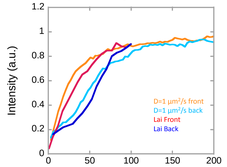


Arp2/3 Oligomer Model Simulated Recovery
D=2 $\mu m^2/s$, K=1.4, D_{olig}=0.5 $\mu m^2/s$, τ_o= 10 s



Scale bar: 2 μm, 3 s/frame

Arp2/3 Oligomer Model



- Lai et al. *EMBO J.* (2008) observe a lag in recovery between the front and back of the lamellipodium
- Model with oligomers captures slow recovery at the back whereas the single cytoplasmic population model has small lag between front and back
- Model suggests single Arp2/3 complexes diffuse quickly to the front, where they bind to the network and then undergo retrograde flow
- Lag in FRAP recovery at the back may represent debranching into oligomers and reannealing. Since oligomers are larger than monomers they would diffuse more slowly than monomers. Oligomers could dissociate and rebind near their previous locations, typically away from the leading edge

6. Conclusions

- Developed simulations of FRAP recovery for CP and Arp2/3 complex at the leading edge using single molecule speckle data
- Good agreement with Kapustina et al. 's experimental capping protein FRAP data is found using a short lifetime as in speckle data and a diffusion coefficient for single CP 3-5 $\mu m^2/s$
- Model accounts for large amount of slowly diffusing capping protein.
- We fit Lai et al.'s experimental Arp2/3 complex FRAP data with a K = 1.4 s^{-1} (bound:cytoplasmic ratio ~ 5) and a diffusion coefficient of 1-2 $\mu m^2/s$, which we expect is due to the size of Arp2/3 complex
- Debranching and reannealing away from leading edge may explain why Arp2/3 intensity recovers more slowly at the back in FRAP experiments

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