

Quantitative analysis of actin assembly and motility during endocytosis in living yeast cells

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Washington University in St. Louis

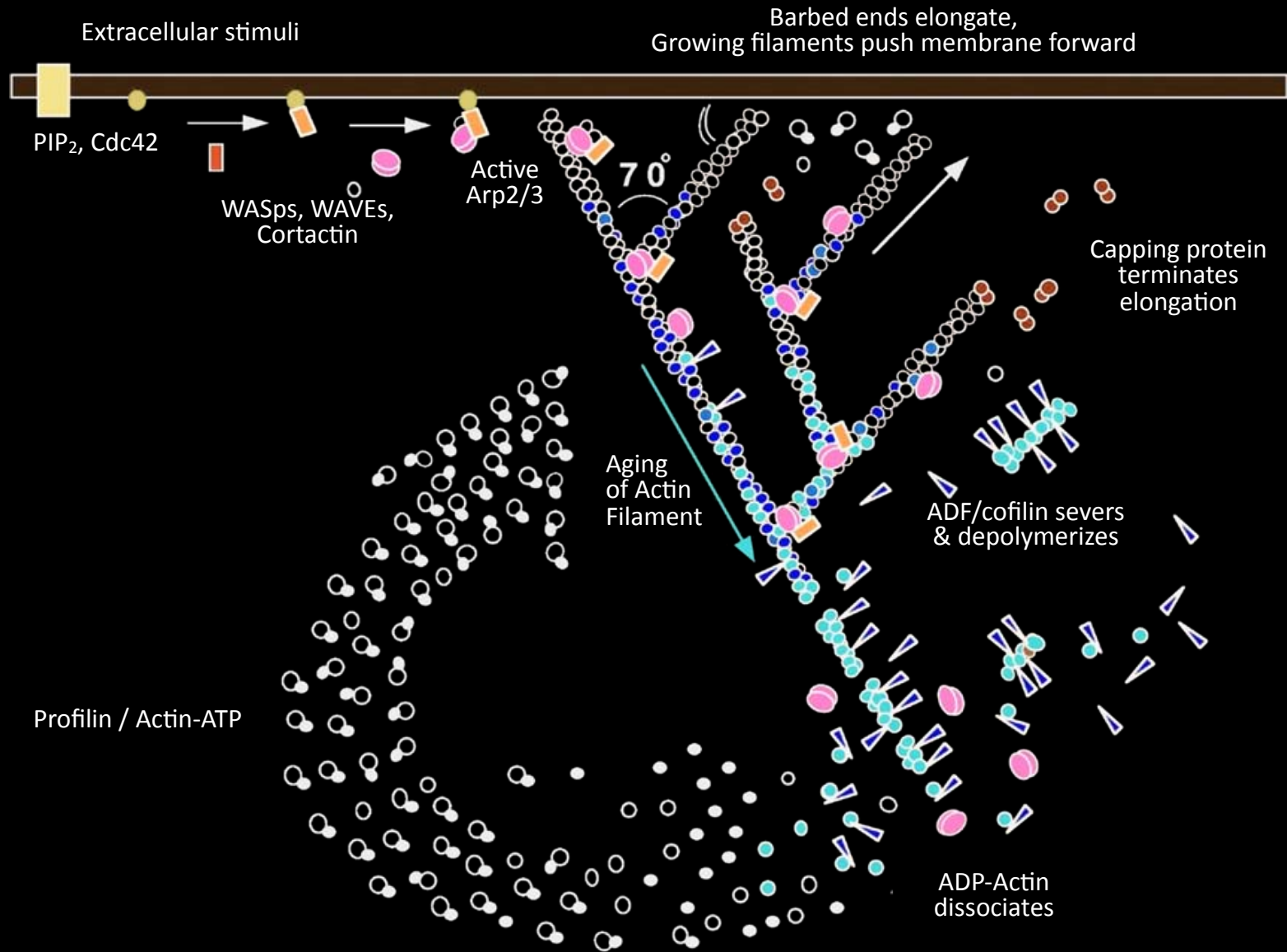


Mathematical Biology of the Cell: Cytoskeleton and Motility
BIRS, Banff
August 2011

Outline

- Introduction
 - Actin Assembly and Motility
 - Arp2/3 and Dendritic Nucleation
- Capping Protein: Yeast Actin Patches
- Multiple Arp2/3 Regulators: Roles and Mechanisms
 - Cortactin in Osteoclasts
 - Yeast Actin Patches

Dendritic Nucleation Model



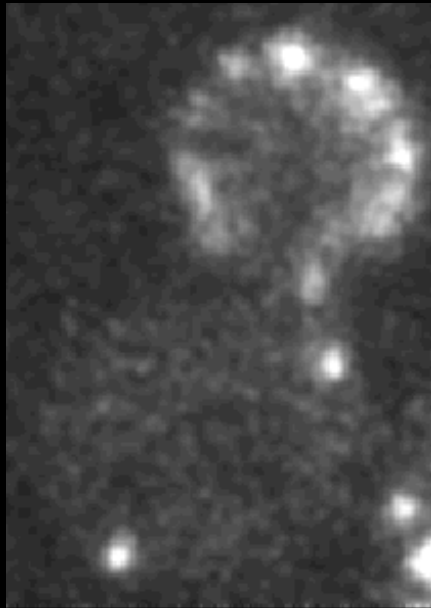
Actin Assembly and Endocytosis



Actin Assembly and Endocytosis in Yeast

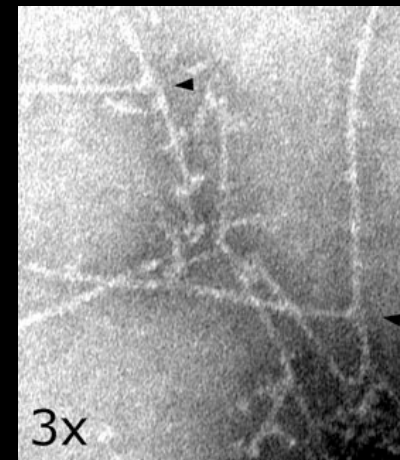
Cortical Actin Patches Mediate Endocytosis

Bud



Mother

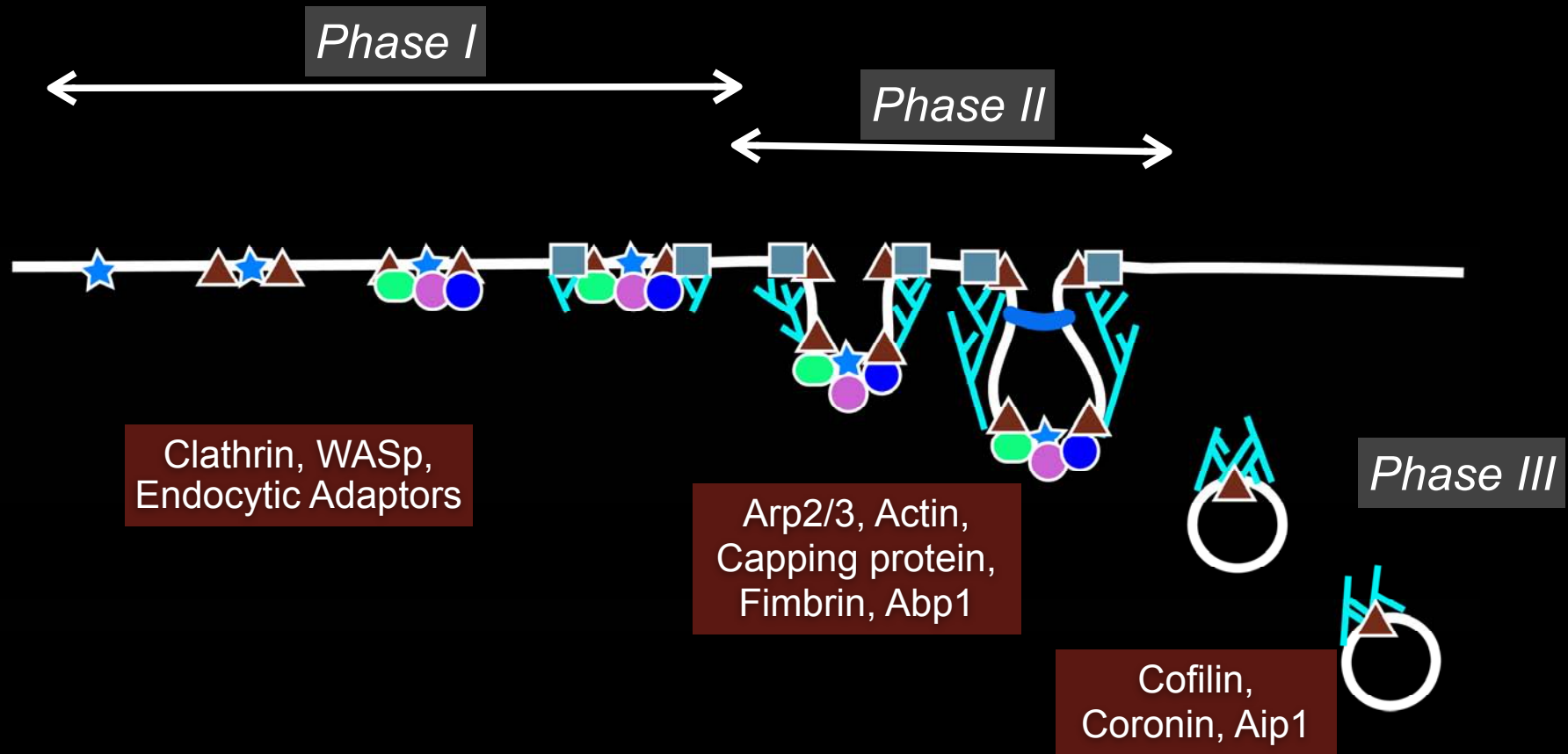
Network of branched
actin filaments



Waddle et al. 1996. *J Cell Biol.* 132:861.

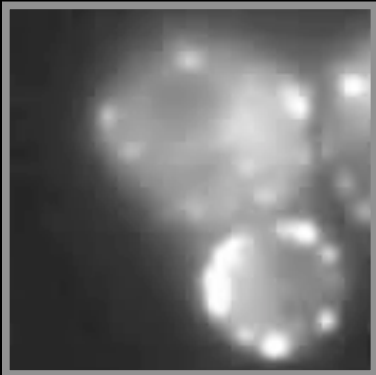
Young et al. 2004. *J Cell Biol.* 166:629.

Actin Assembly and Endocytosis in Yeast



Contributions from labs of David Drubin, Sandy Lemmon, Liza Pon, Barbara Winsor and Rong Li.

Molecular Composition over Time



Patch protein-GFP

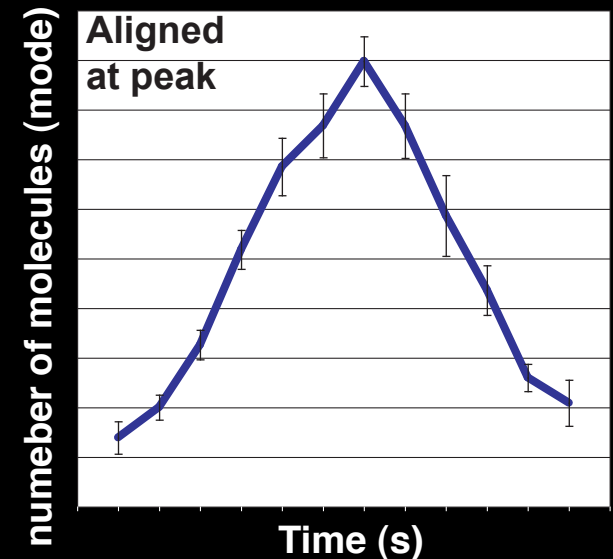
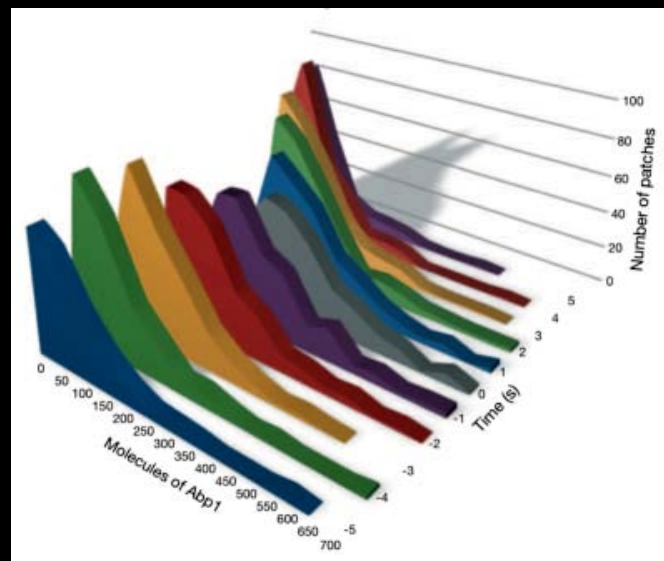
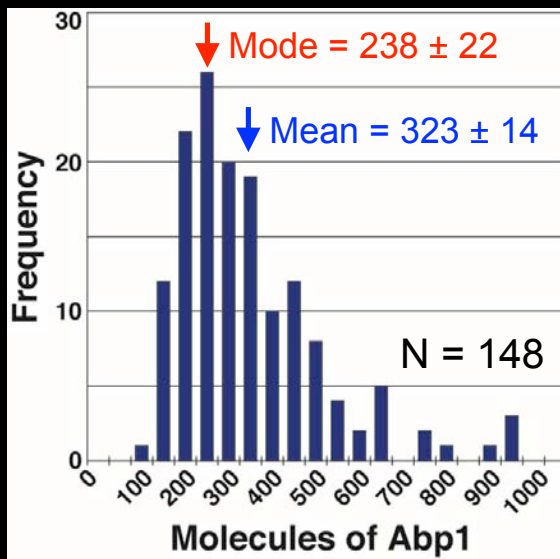


Cse4-GFP

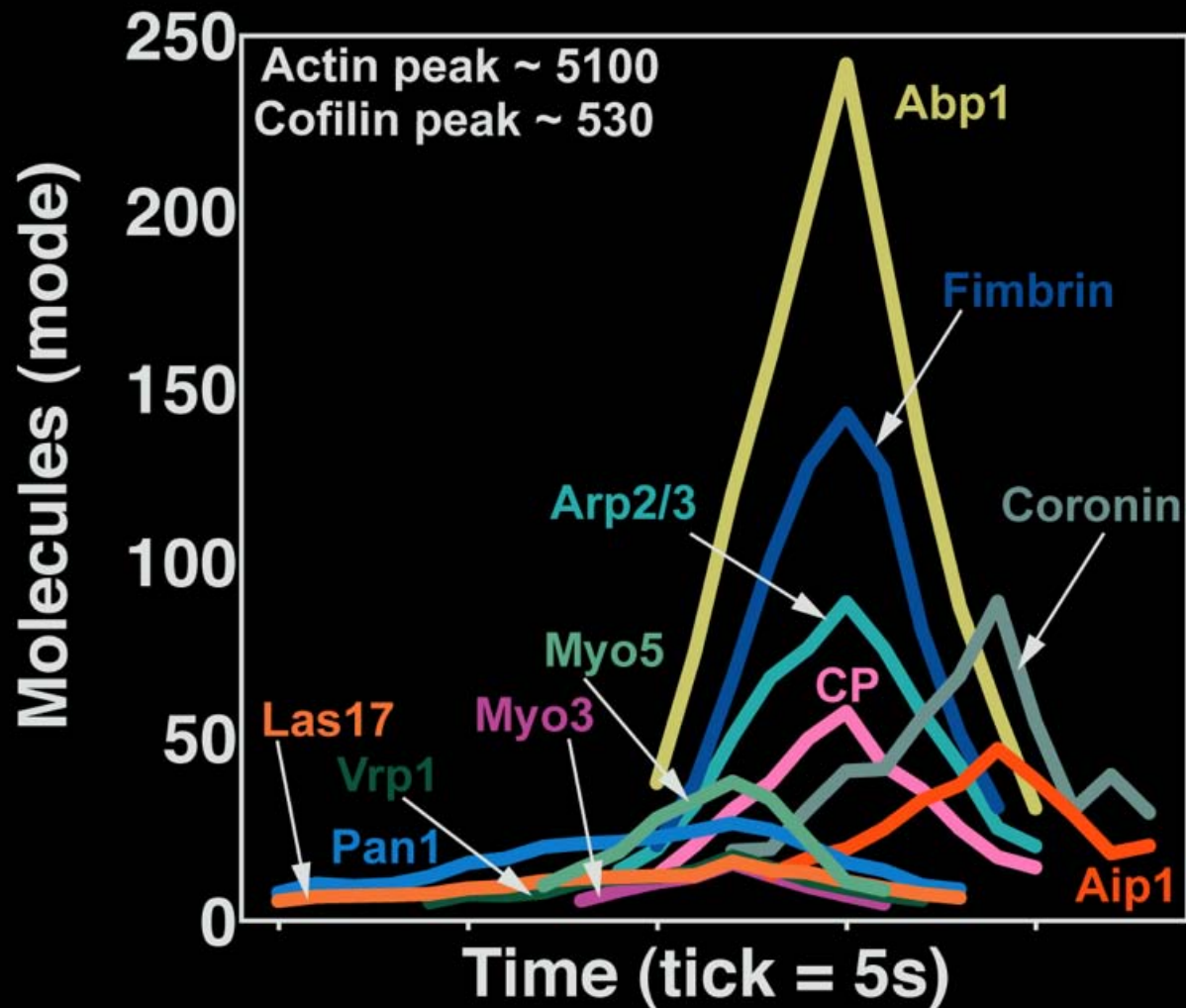
- Digital Fluorescence movies of cells expressing GFP-labeled dendritic nucleation proteins
 - Expression from endogenous locus
 - Function of the GFP fusion
 - ▶ Rescue null mutation
 - ▶ Proper assay
 - ▶ Function in combinations of mutations
- Convert to numbers of molecules
 - Background subtraction
 - Standards in cells
 - ▶ Kinetochores Cse4-GFP in yeast expressed from chromosomal locus (32, now 109)
 - ▶ Bacterial flagellar protein MotB (22)
- Hundreds of patches in wildtype and mutant cells

Data treatment

- Intensity data for every patch: Number of molecules vs time
- Align curves from different patches at the time of peak molecule number
- Histogram of values from all patches at each time point
- Calculate mode at each time point
 - mean of the bootstrapped half-range modes (Hedges and Shah, 2003)
- Plot Mode vs Time

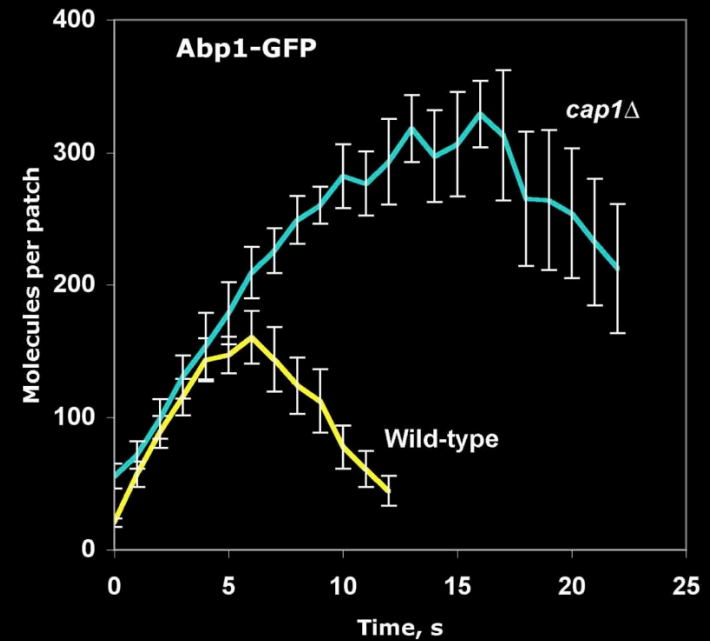
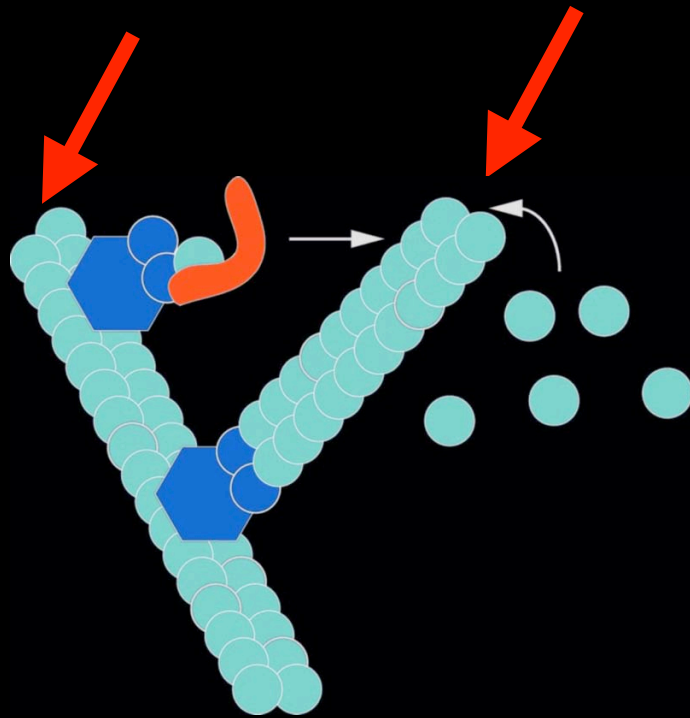


Molecular Composition of Patches over Time



Includes timing information from Sun et al. 2006. Dev Cell. 11:33.

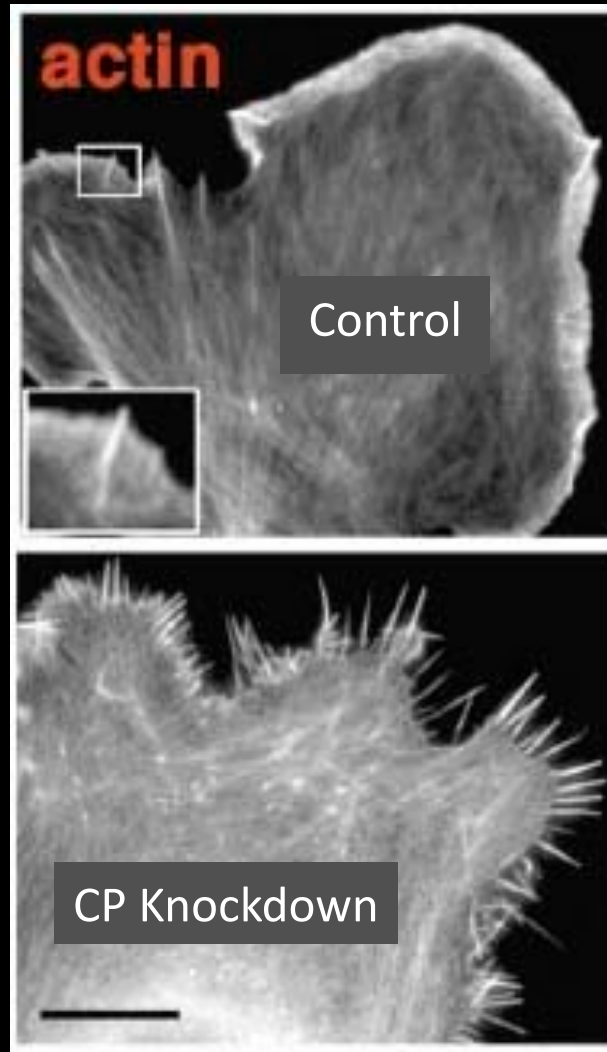
Capping Protein Mutant: F-Actin Marker



- Consistent with Capping Protein Limiting Actin Assembly

Loss of CP -> Loss of Arp2/3 Network

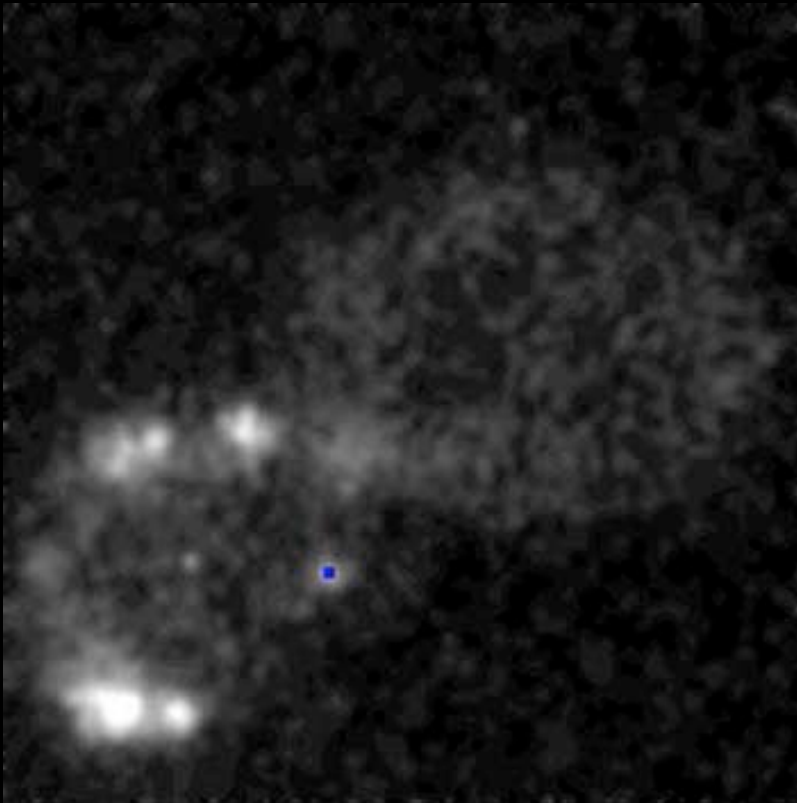
Lamellipodia in B16 Cells



Mejillano et al. 2004. Cell. 118:363.

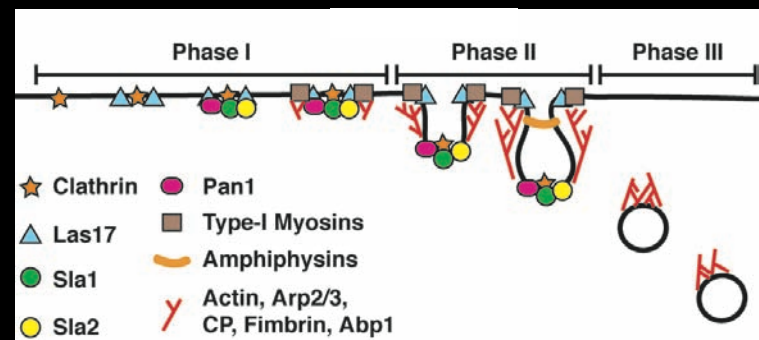
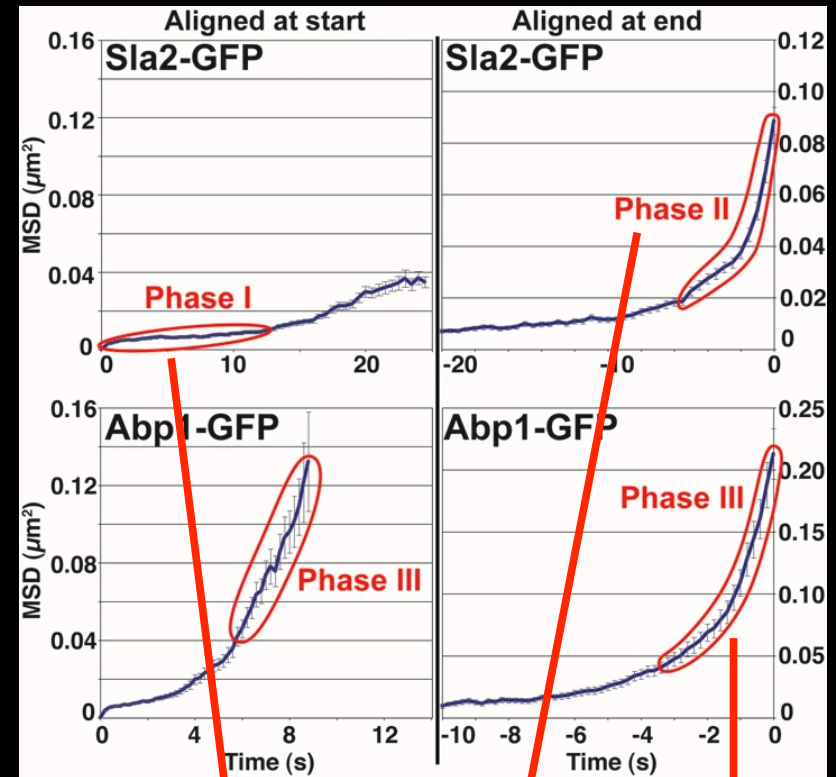
Analysis of Patch Motion

- Computer tracking program
 - Detects each patch in every frame
 - Links them together over time to create tracks
 - Analysis of hundreds of tracks in multiple isolates



Motion Data Analysis

- Mean Squared Displacement (MSD) vs time
 - Patch Curves Aligned at Start vs End
- Fraction of patches that make transitions of movement
- Time that patches remain in a given phase of movement
- Isolate the character of particles undergoing certain phases of motion



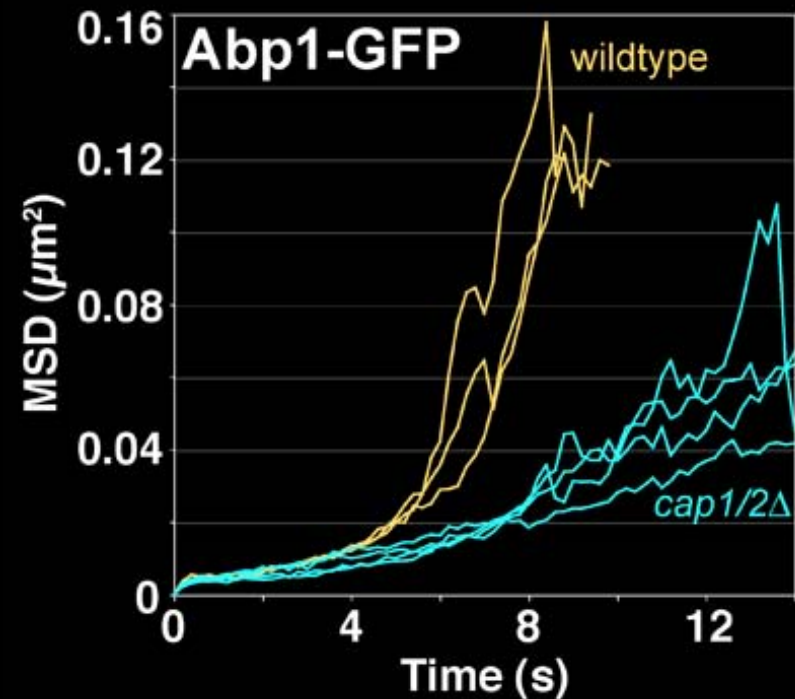
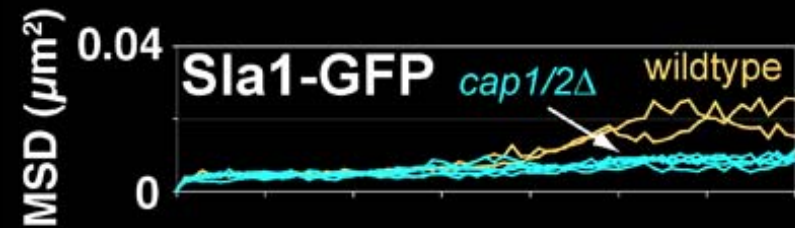
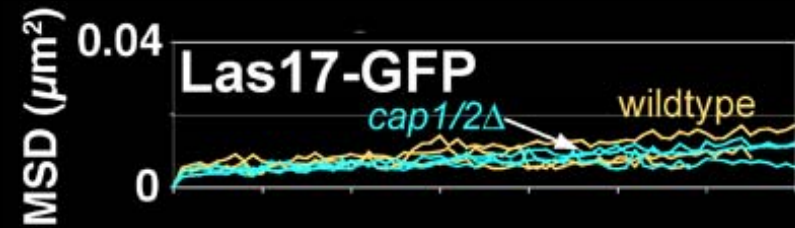
Effect of Loss of CP on Patch Movement

GFP-Fusion Marker

Early

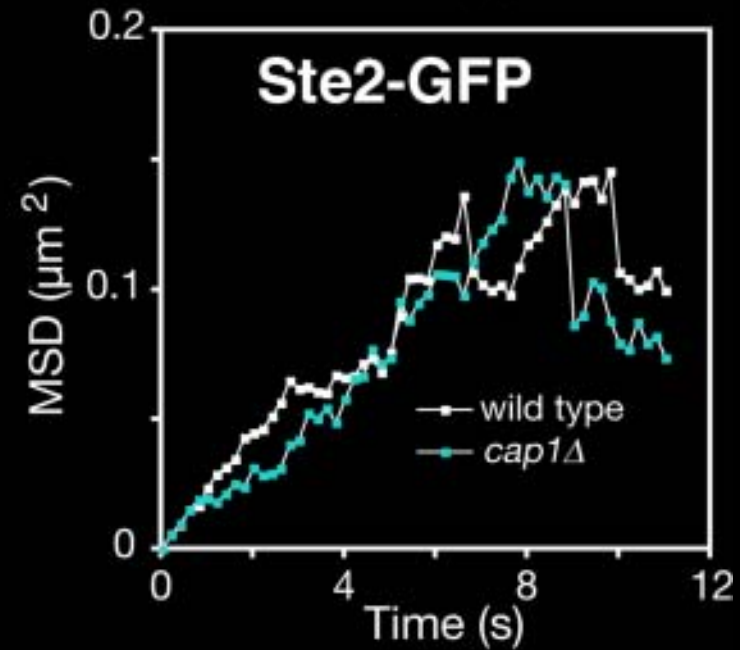
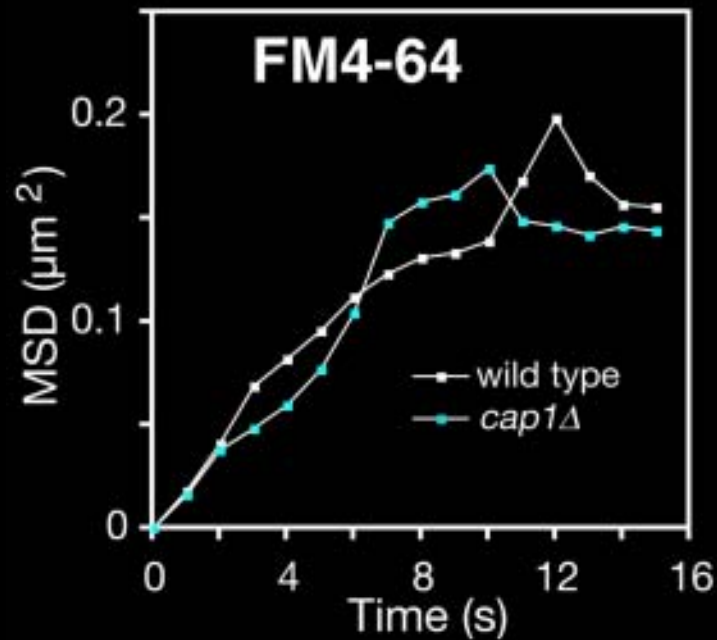
Middle

Late



- Decreased Movement Away from the Membrane

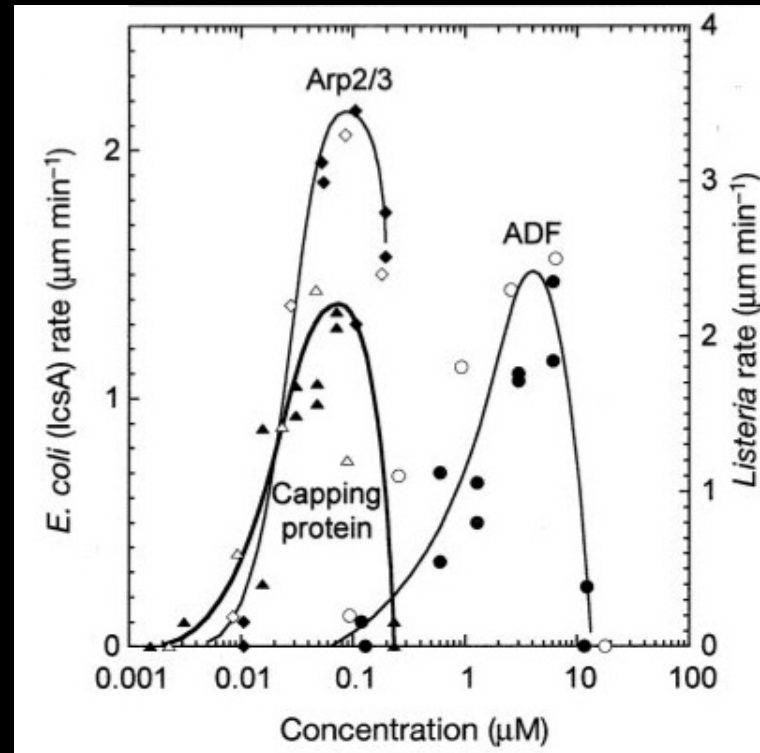
Vesicles Moving from Plasma Membrane to Vacuole



- No Effect of Loss of CP

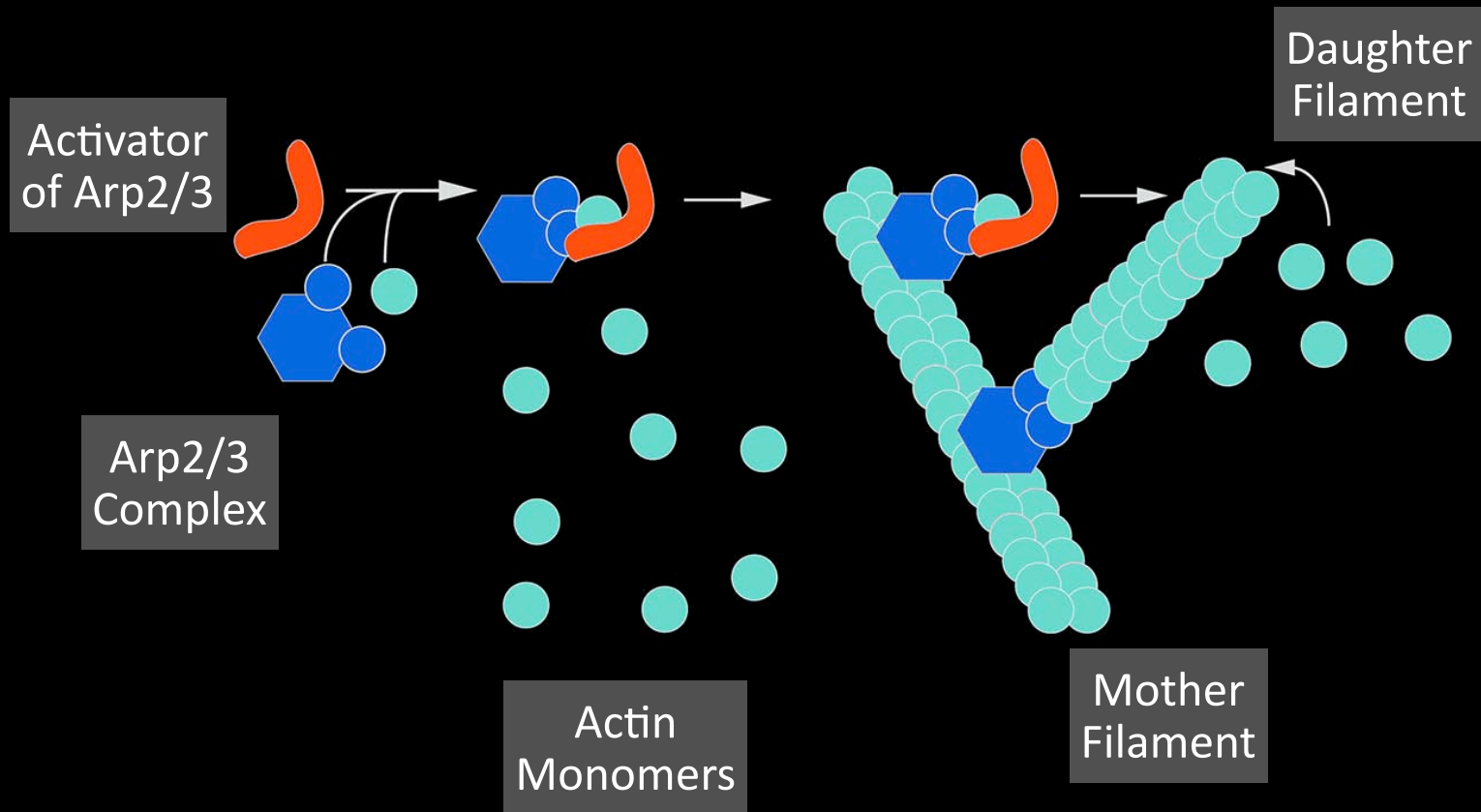
CP is Necessary for Actin Assembly-based Movement

Reconstitution System
for Bacterial Movement



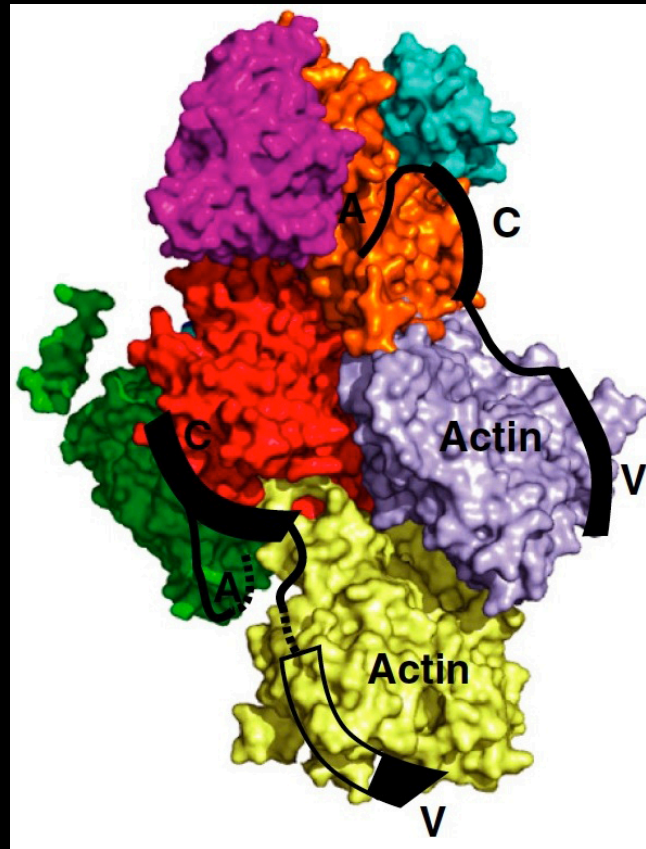
Loisel et al. 1999. Nature. 401:613.

Arp2/3 Creates a New Branched Filament



- Individual Roles for Multiple Regulators?
- Function at Different Places and Times?

Two Nucleators Bind One Arp2/3



Arp2/3 complex is bound and activated by two WASP proteins

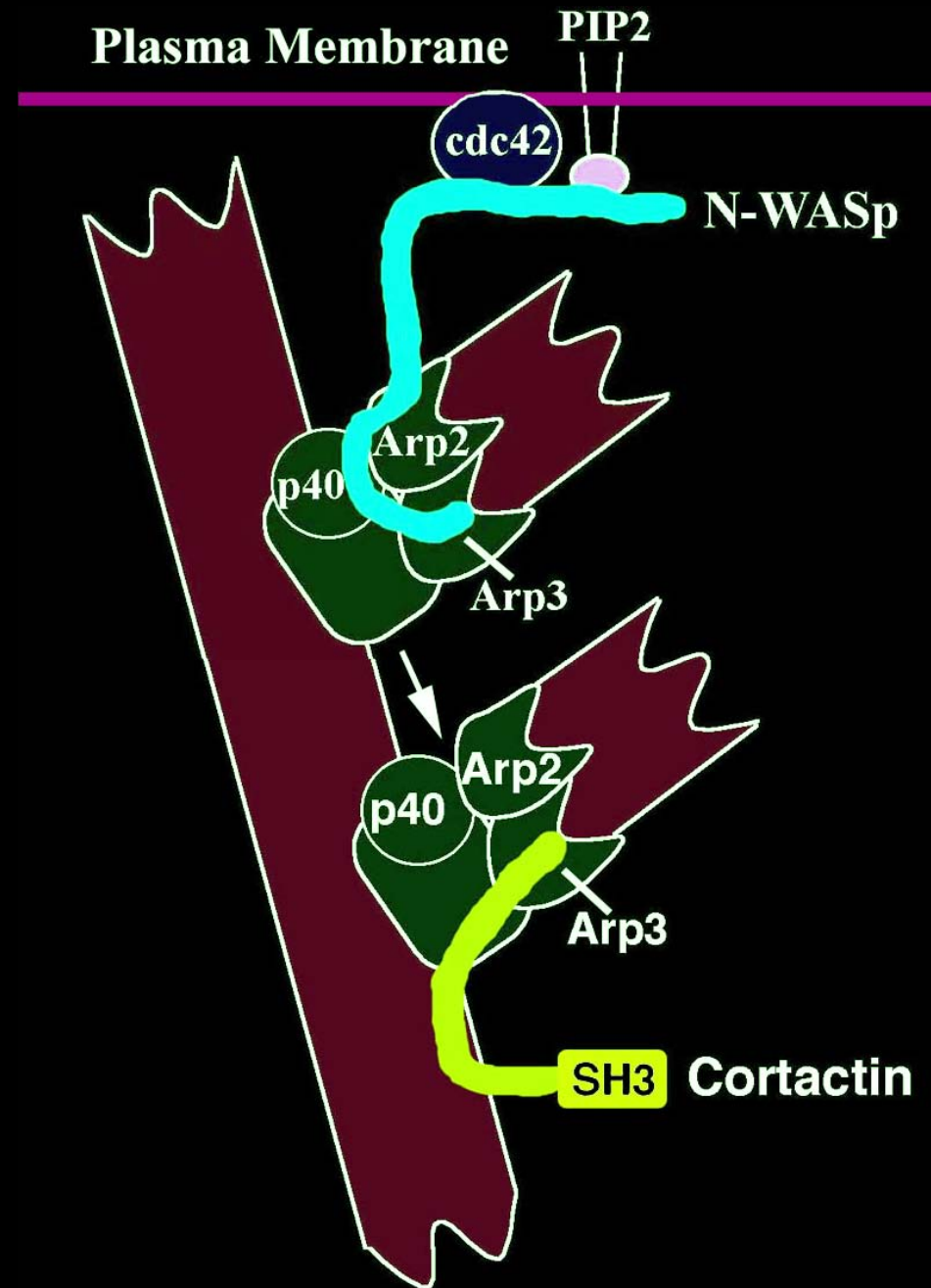
Shae B. Padrick^a, Lynda K. Doolittle^a, Chad A. Brautigam^a, David S. King^b, and Michael K. Rosen^{a,1}

^aHoward Hughes Medical Institute and Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390; and ^bHoward Hughes Medical Institute Mass Spectrometry Laboratory and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202

Proc Natl Acad Sci U S A. 2011. Jun 15 Epub ahead of print.

Model for N-WASp and Cortactin

Cortactin Replaces N-WASp as Arp2/3 is Pushed Away from the Membrane

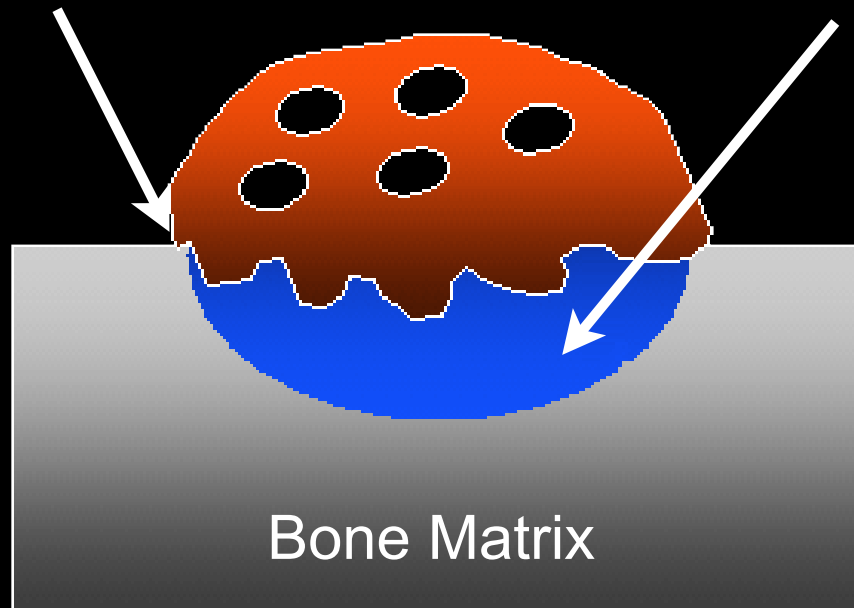


Weaver *et al.* 2002. *Curr Biol.* 12:1270-1278.

Osteoclast: Bone Resorption

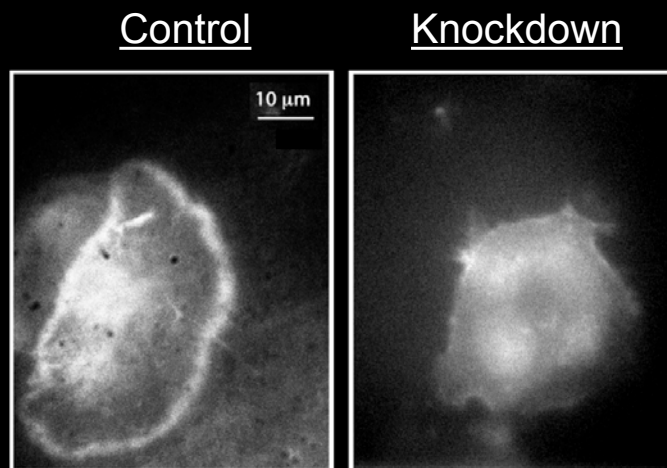
Sealing Zone:
Integrin, Actin

Acid,
Proteases



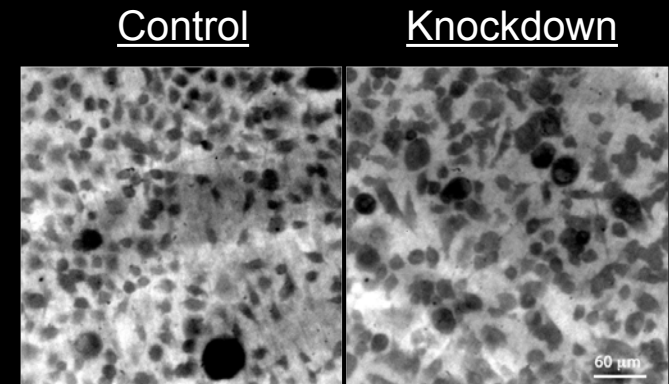
Bone Matrix

Cortactin-depleted Osteoclasts on Bone

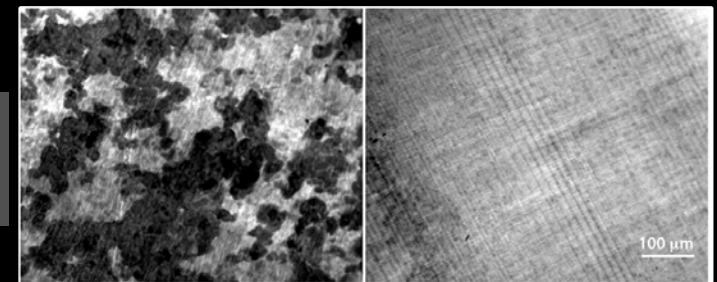


F-Actin
Single Cells

Nonspecific
Stain for Cells

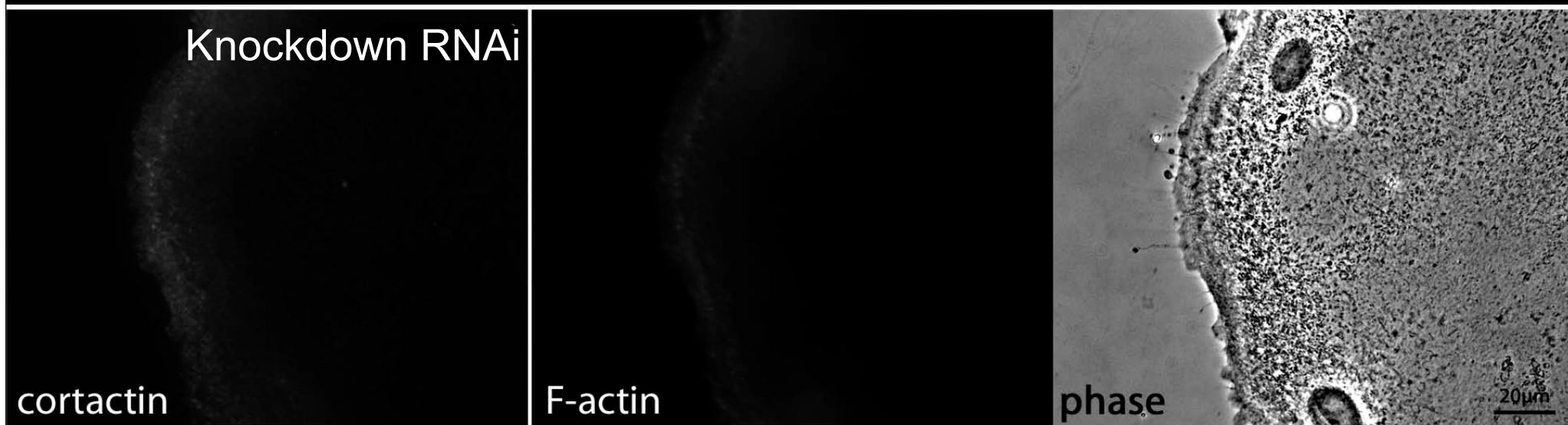
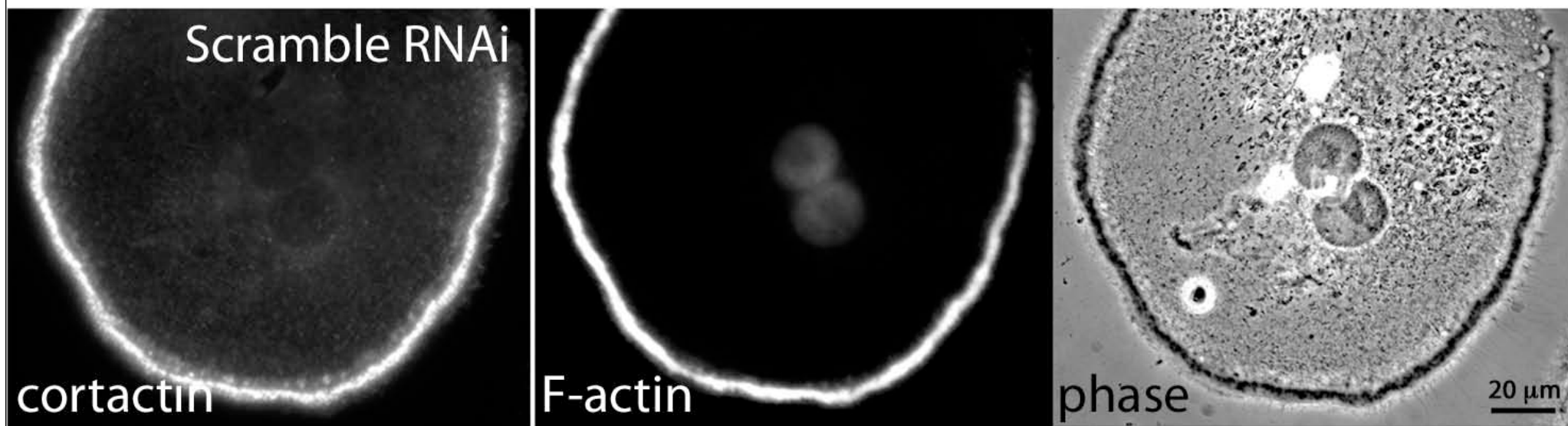


Stain for Bone
Resorption Pits



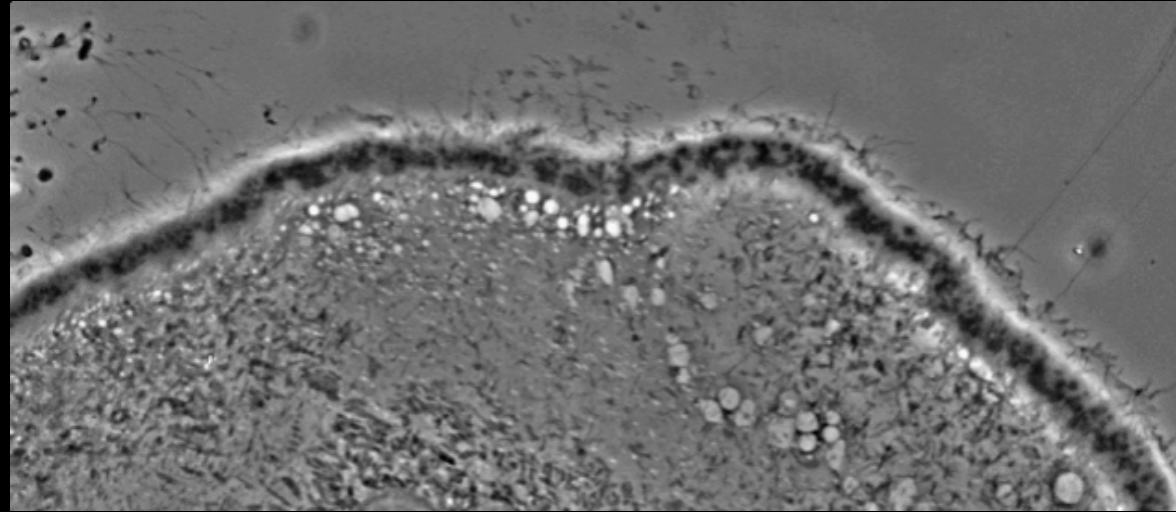
Tehrani et al. 2006. *Mol Biol Cell*. 17:2882.
Tehrani et al. 2007. *Proc Natl Acad Sci U S A*. 104:11933.

Cortactin-depleted Cells on Glass

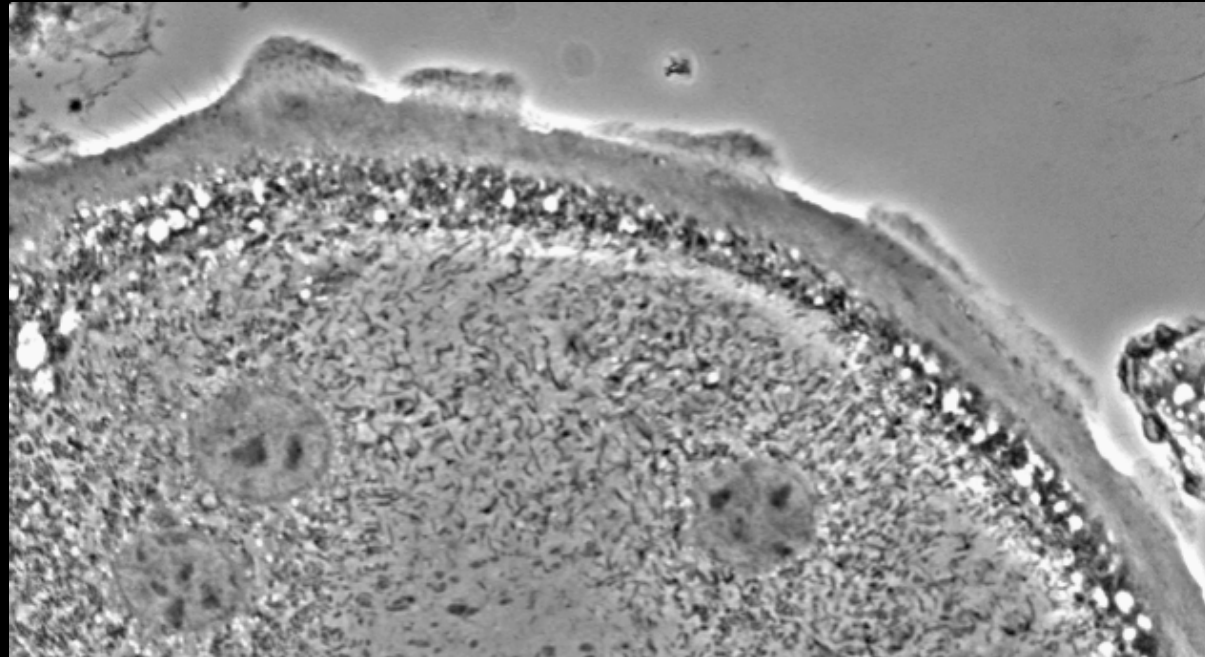


Podosome Dynamics

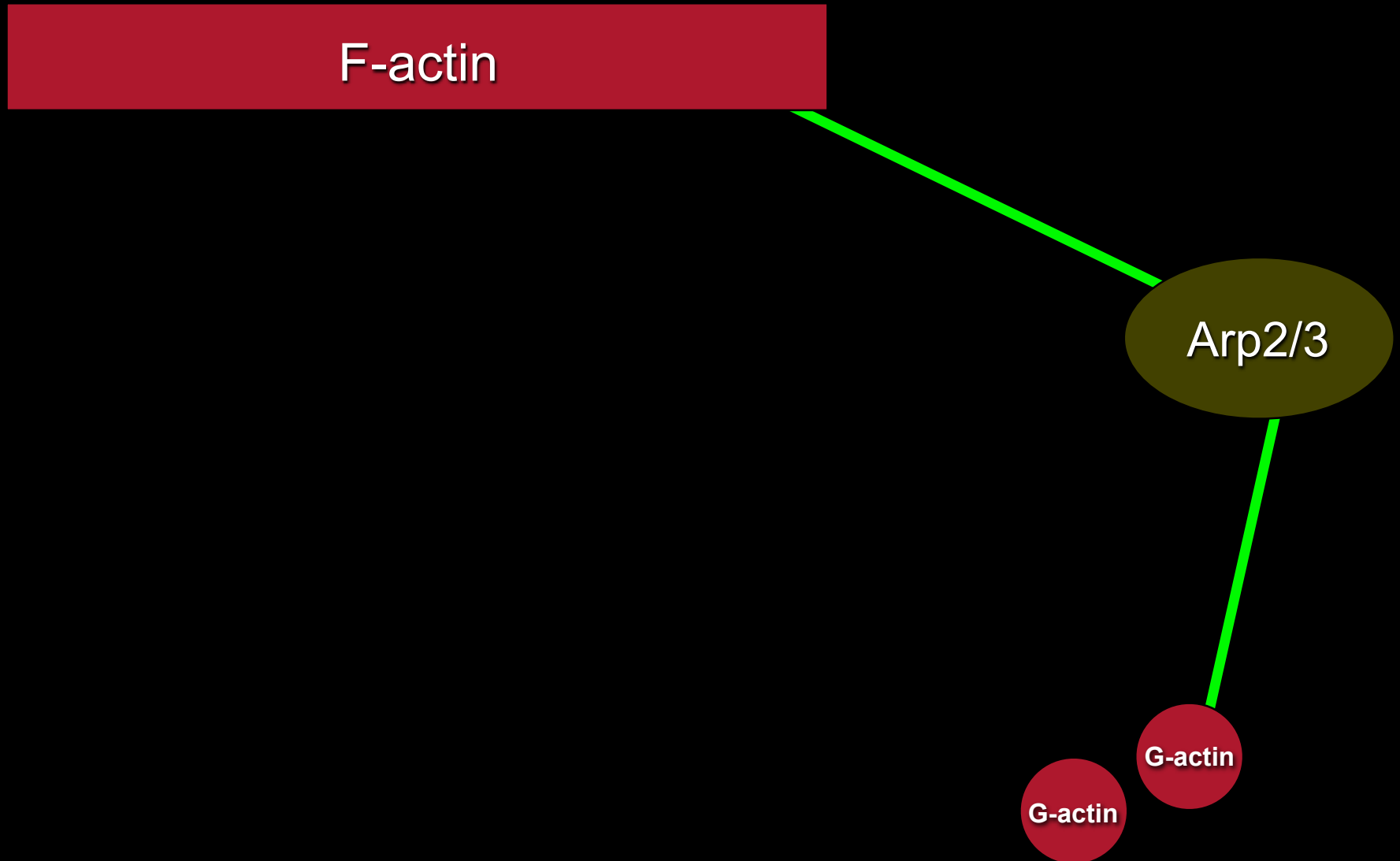
Control



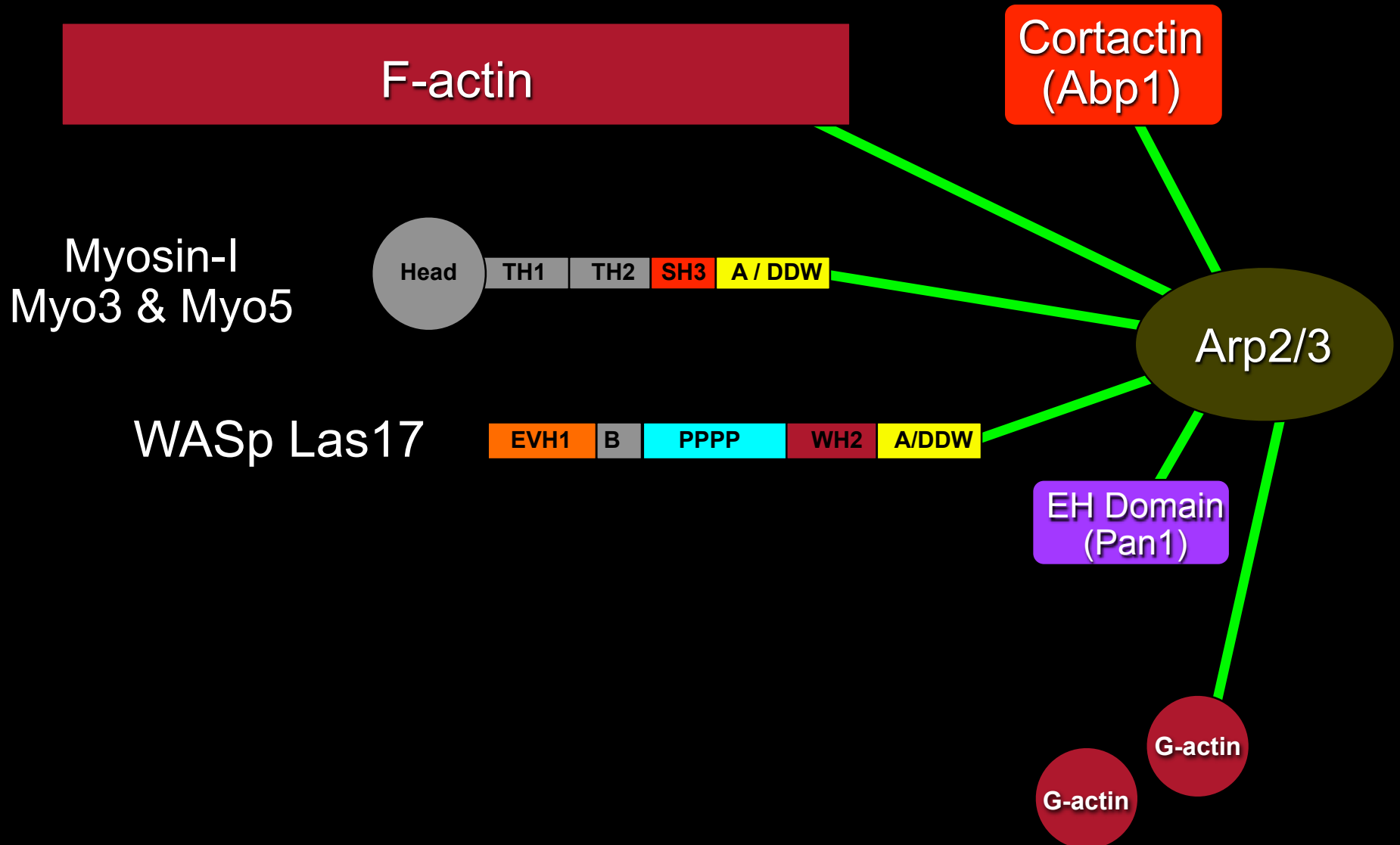
Cortactin
Knockdown



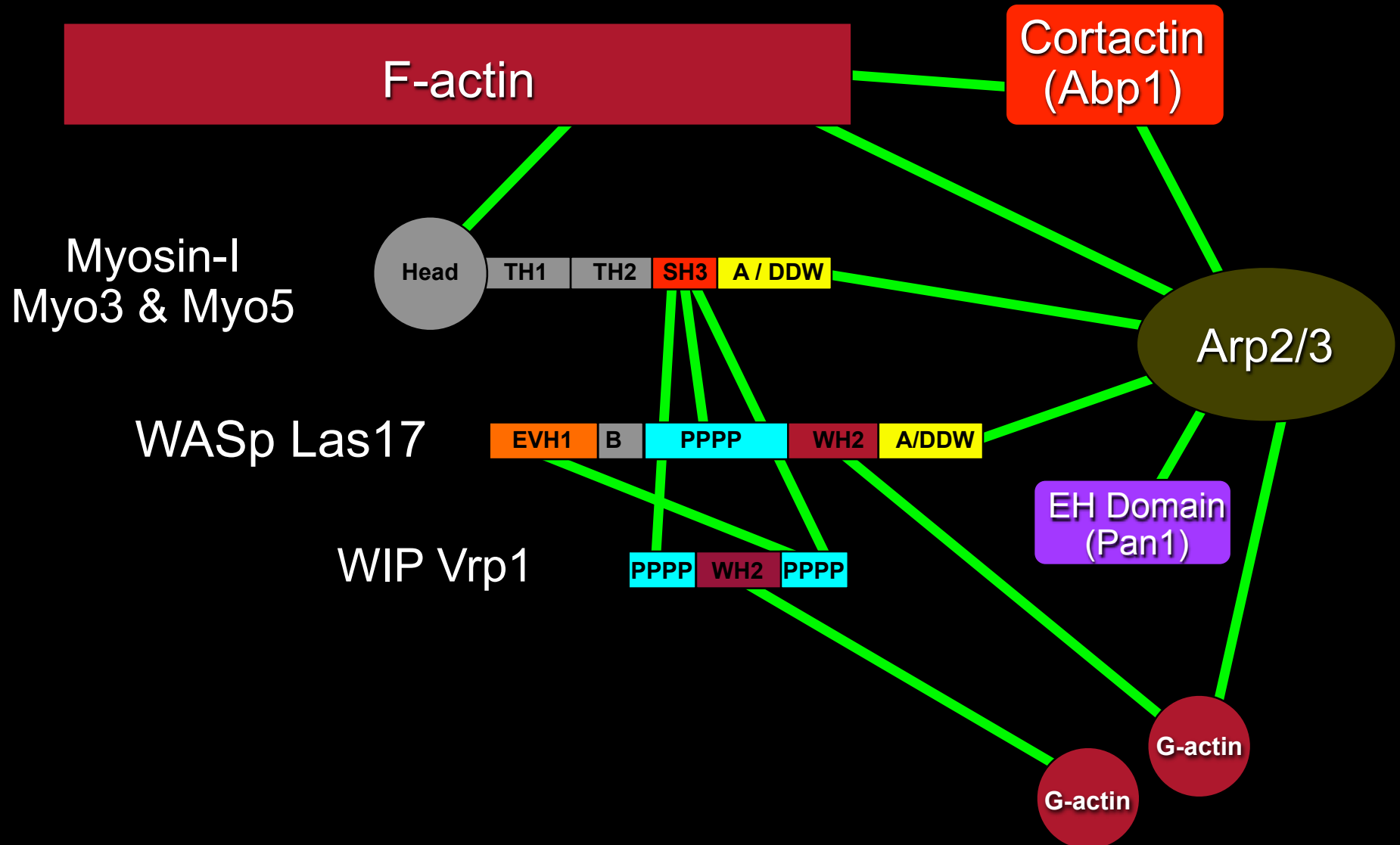
Complexity in Potential Regulators of Arp2/3 in Yeast



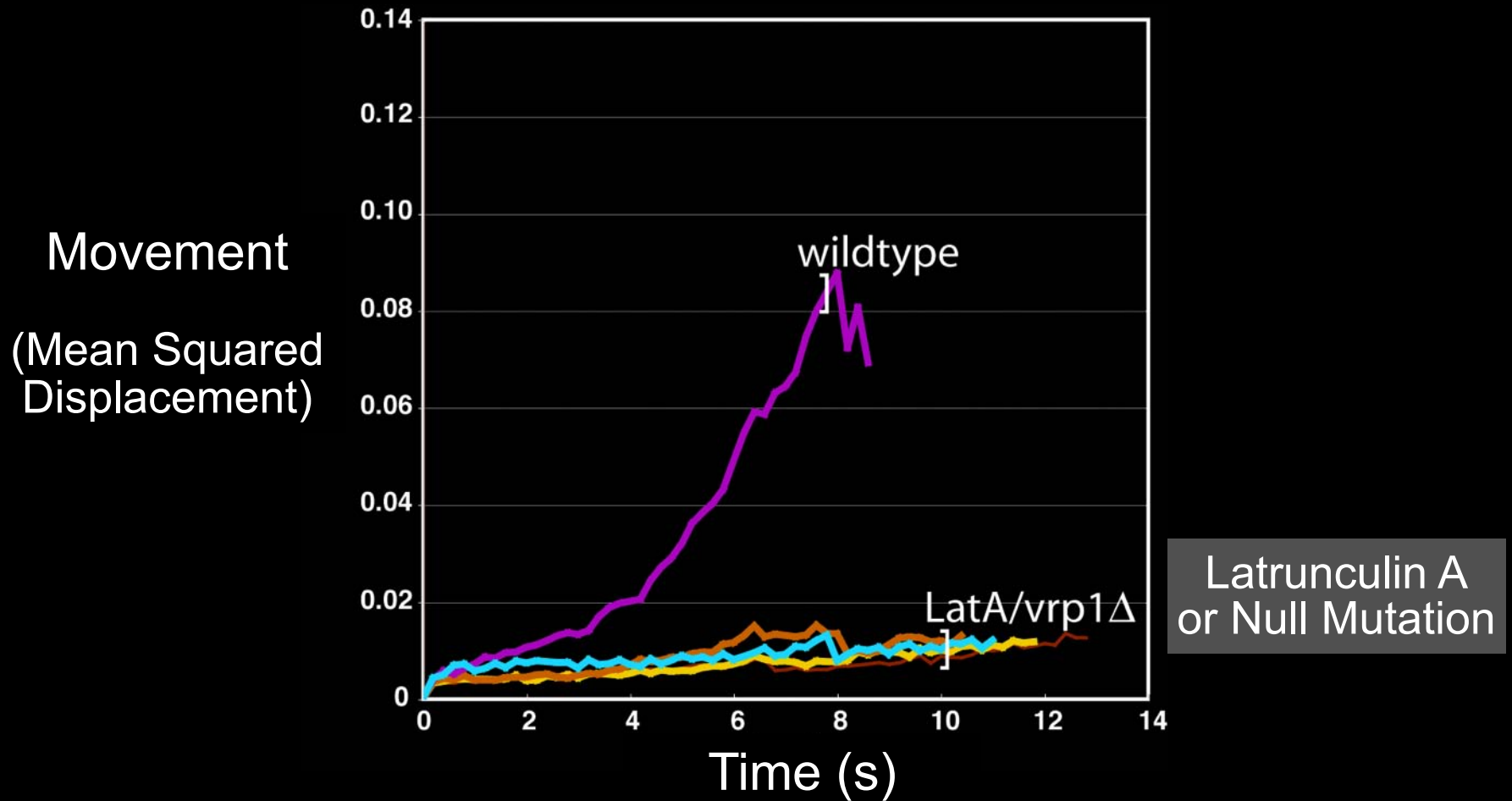
Complexity in Potential Regulators of Arp2/3 in Yeast



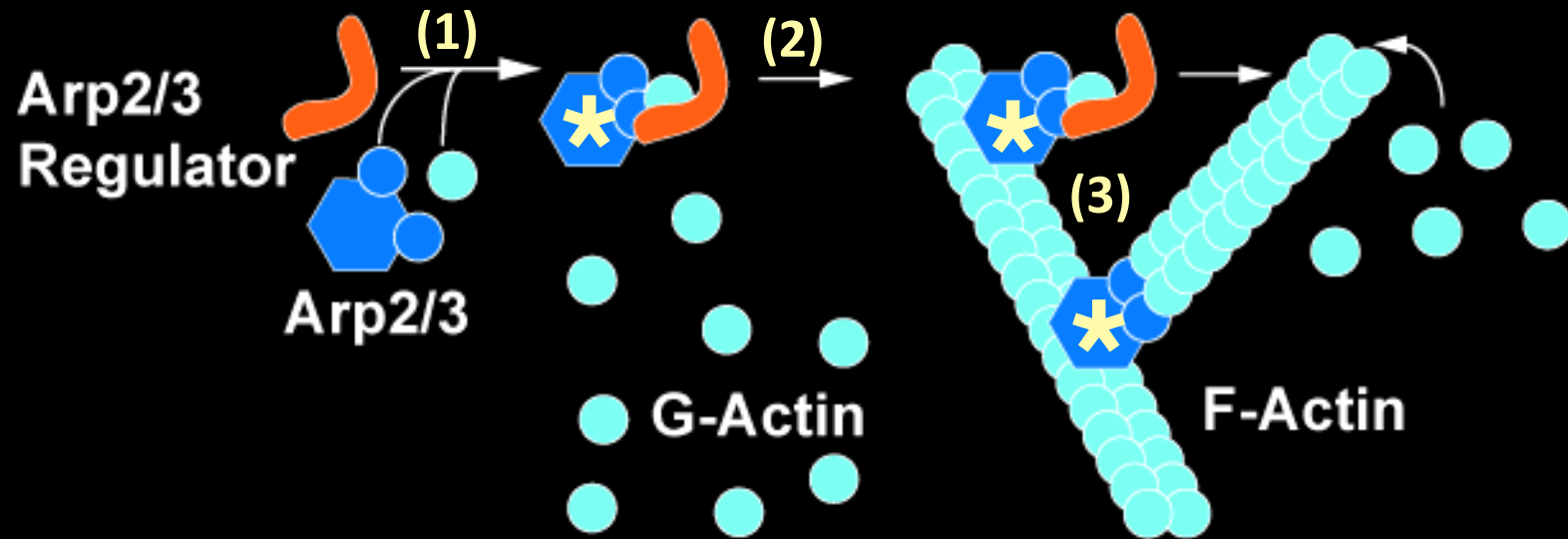
Complexity in Potential Regulators of Arp2/3 in Yeast



WASp-Interacting Protein (WIP/Vrp1) is Crucial



Potential Mechanisms for Arp2/3 Regulator Function



1) Recruitment of Arp2/3 to the site of actin assembly

Measure Levels of Arp2/3

2) Activation of Arp2/3 to nucleate new filaments

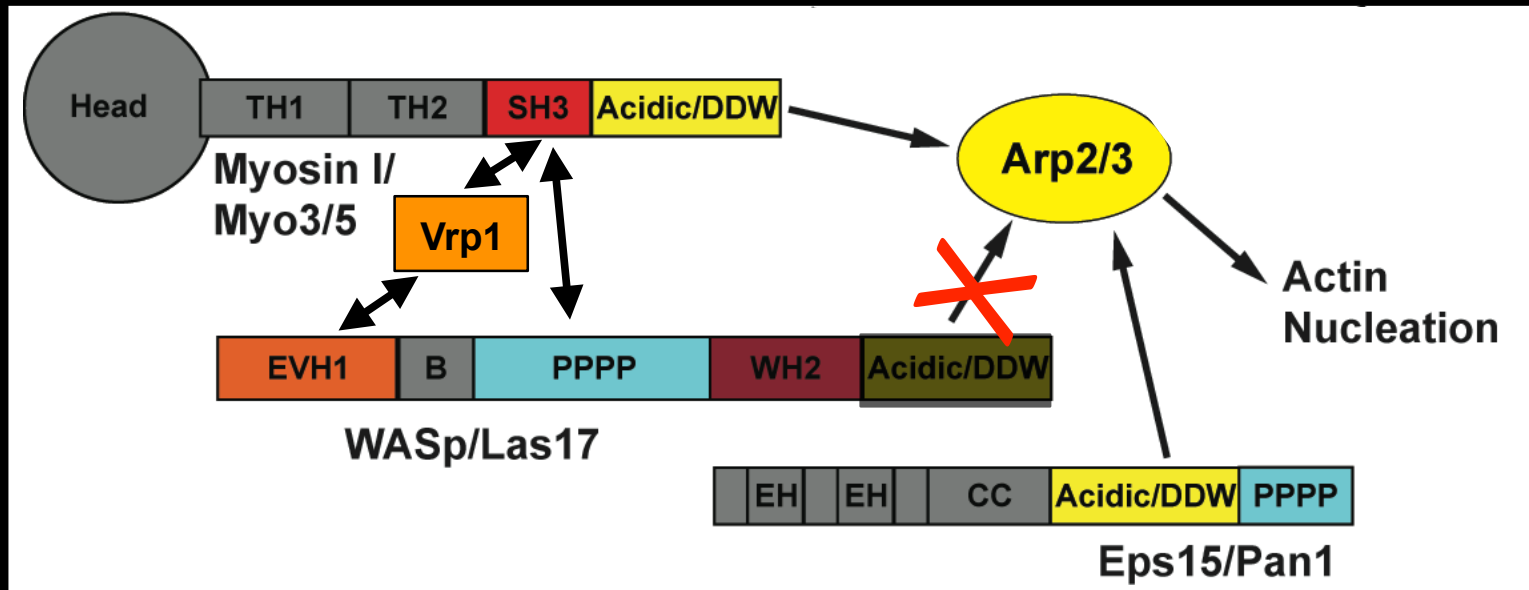
Measure Levels of Capping Protein (Barbed Ends) and Abp1 (F-actin)

3) Network stability and architecture

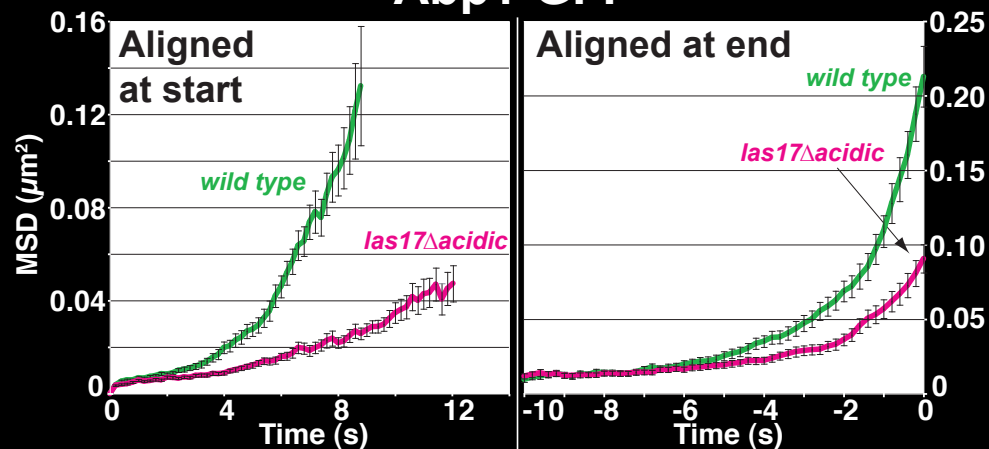
Measure Rates of Patch Assembly & Disassembly

Measure Ratios of the Network Components

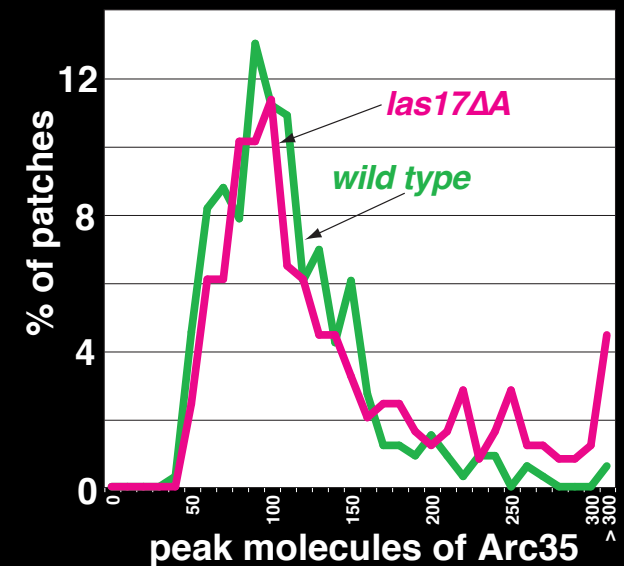
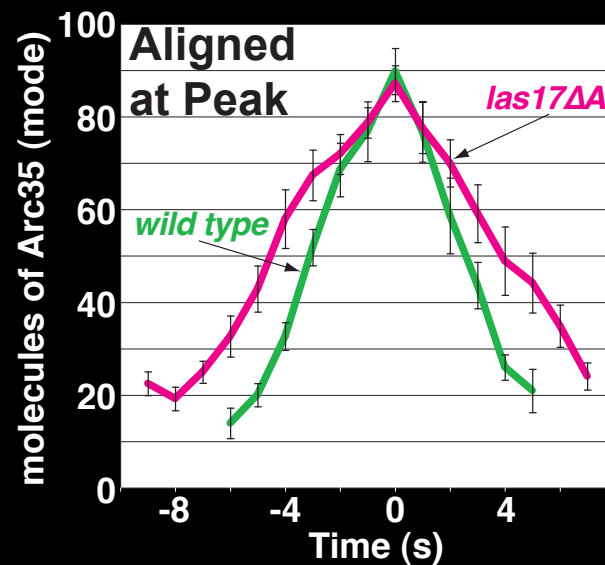
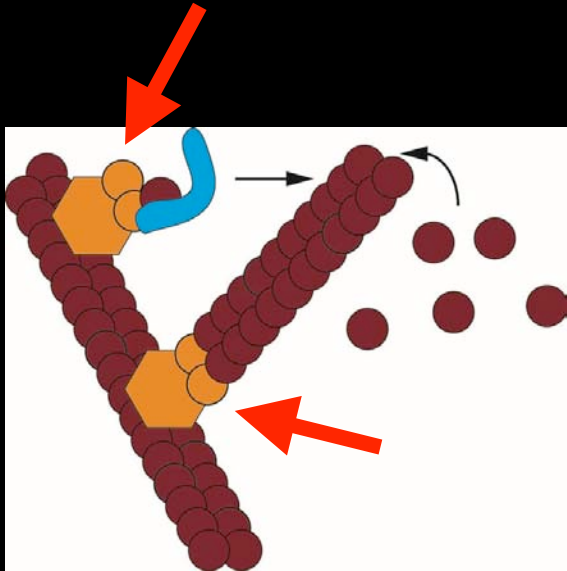
How Does Loss of Arp2/3 Binding by WASp/Las17 Affect Actin Patch Assembly and Movement?



Abp1-GFP

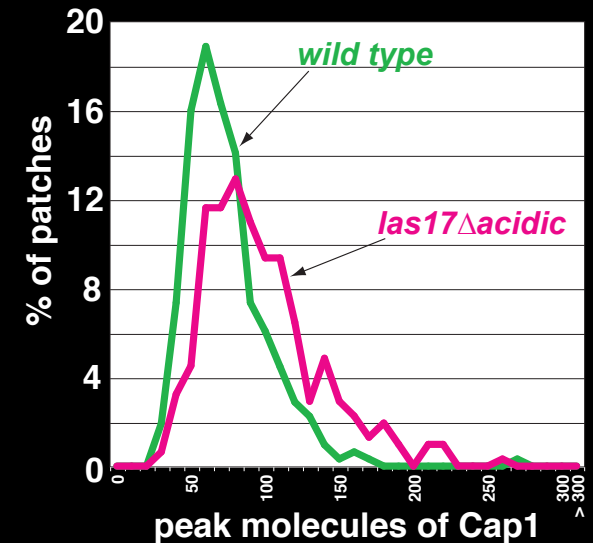
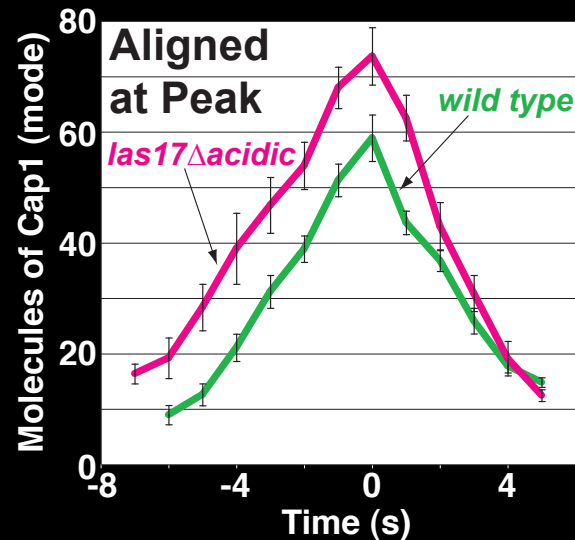
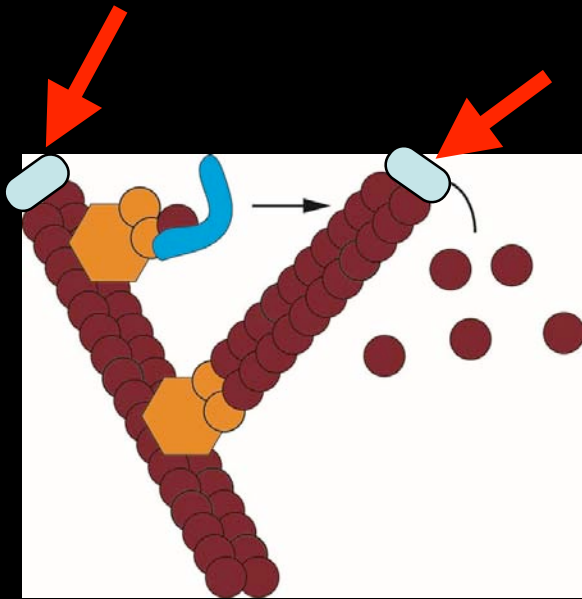


Arp2/3 levels in WASp/Las17 Δ acidic patches



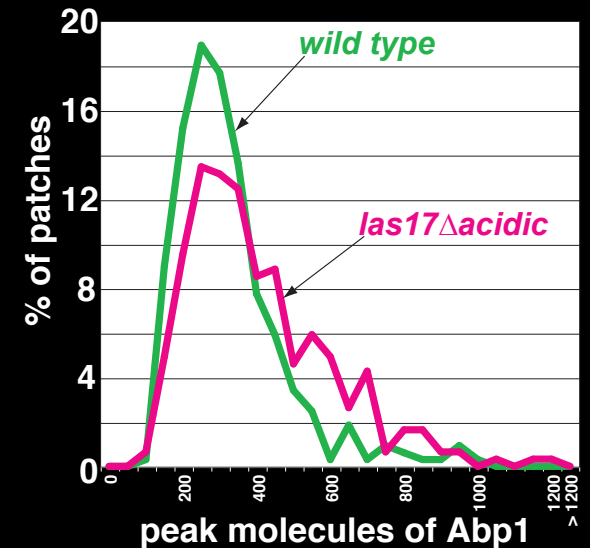
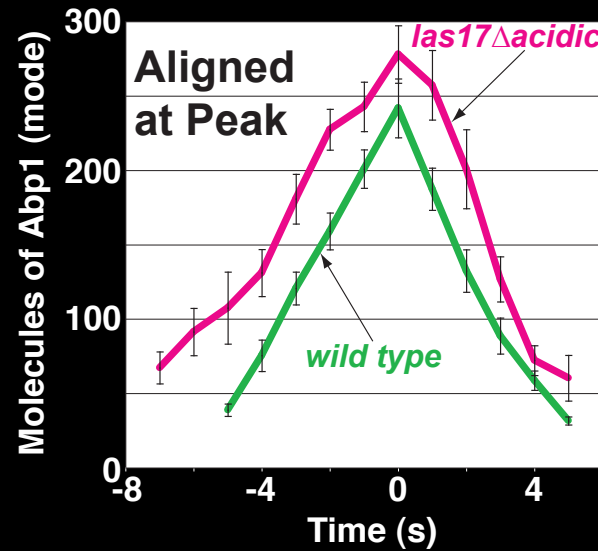
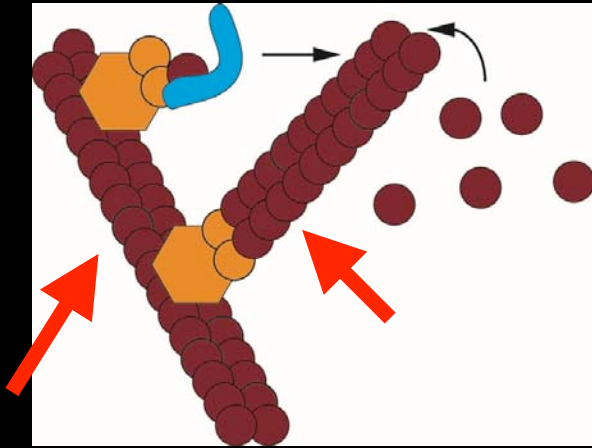
- Peak amount of Arp2/3 recruited is unchanged
∴ Motility defect is not due to a failure to recruit Arp2/3
- Slower assembly and disassembly phases

Capping Protein (Barbed End) Levels in WASp/Las17 Δ acidic Patches



- The amount of CP recruited is increased ... ?
 - ∴ Motility defect not due to insufficient barbed ends
- Assembly: No Δ Rate, \uparrow Duration
- Disassembly: \uparrow Rate, \uparrow Duration

Abp1 (F-actin) in WASp/Las17 Δ acidic patches

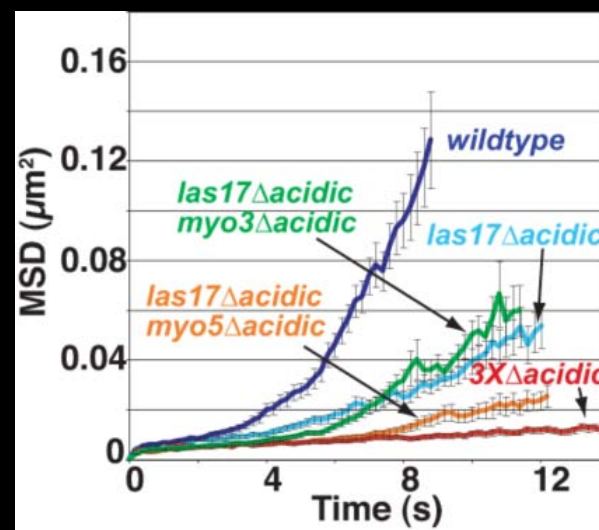
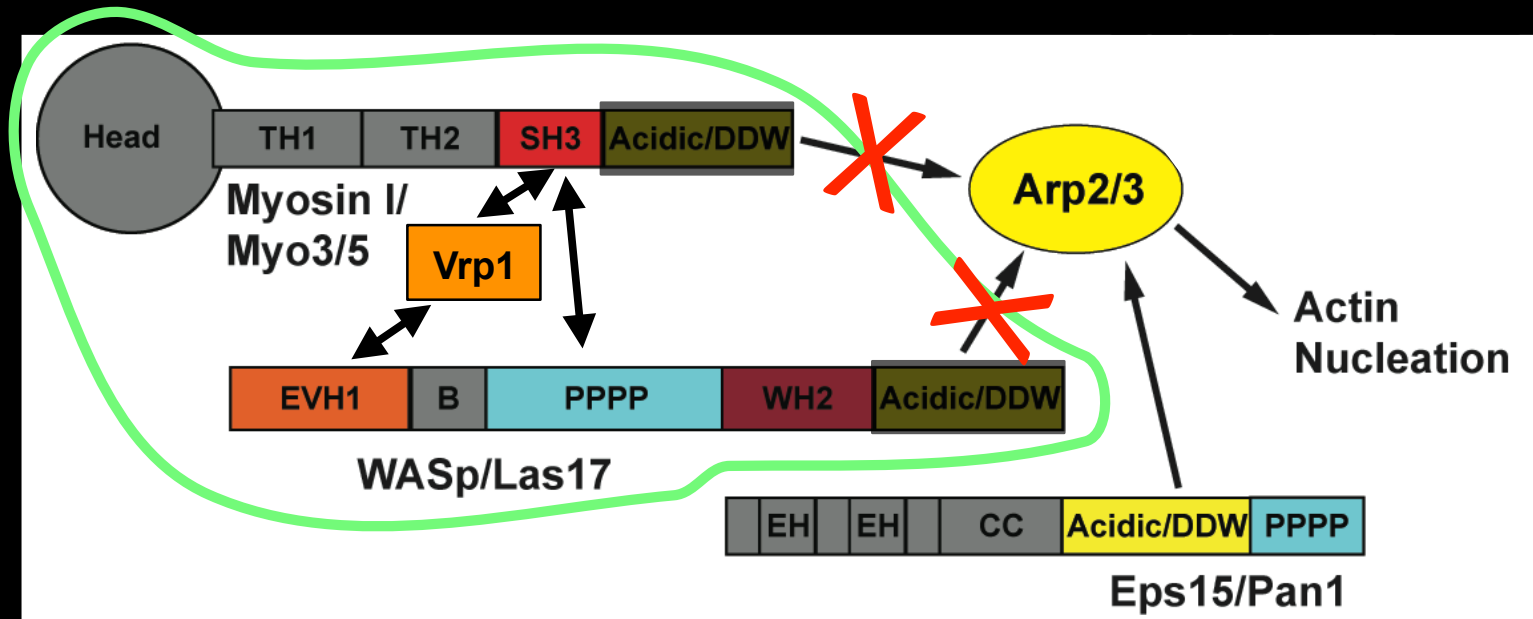


- The amount of Abp1 recruited is slightly increased
∴ Motility defect not due to insufficient F-actin
- Assembly: Rate unchanged, Duration increased
- Disassembly: Rate increased, Duration unchanged

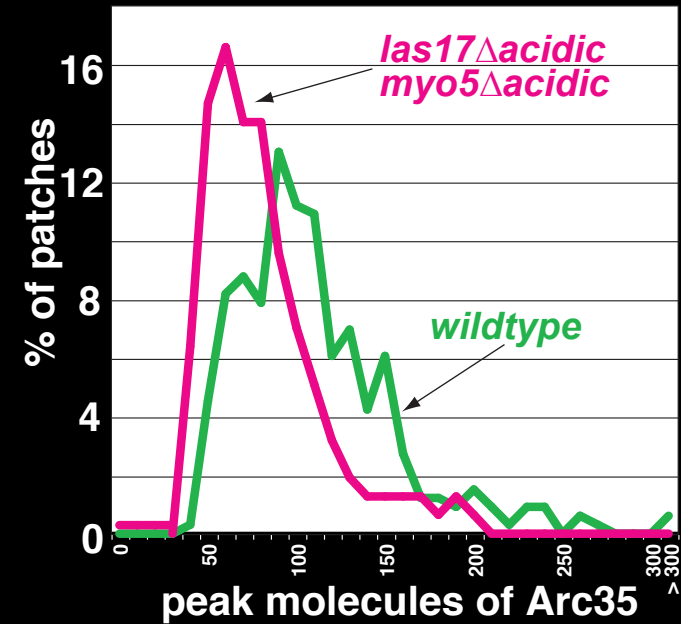
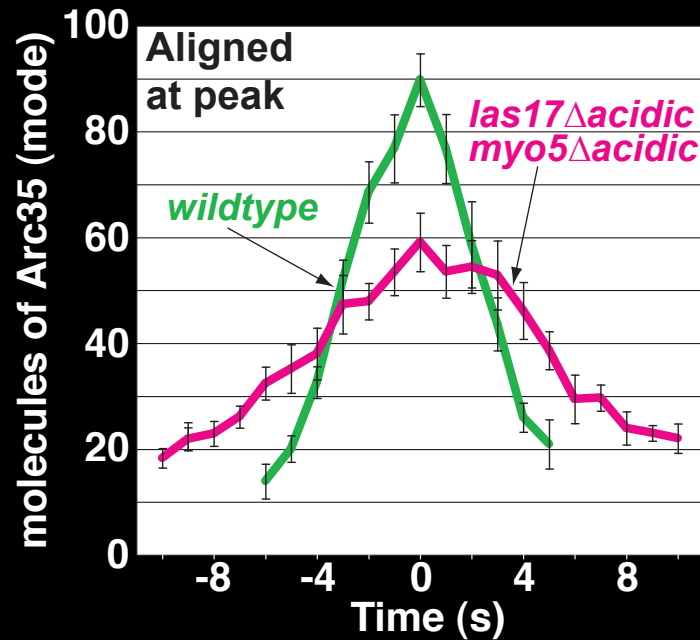
Summary for WASp/Las17 Mutant

- Cause of Moderate Motility Defects?
 - No ↓ recruitment Arp2/3
 - Modest ↓ rate of Arp2/3 recruitment
 - No ↓ nucleation actin filaments
 - ▶ ↑ barbed ends and F-actin ... Why?
- Defect in Architecture of Filament Network?

Combining Mutations in WASp/Las17 Δ with Ones in Myosin-I (Myo3 & Myo5)

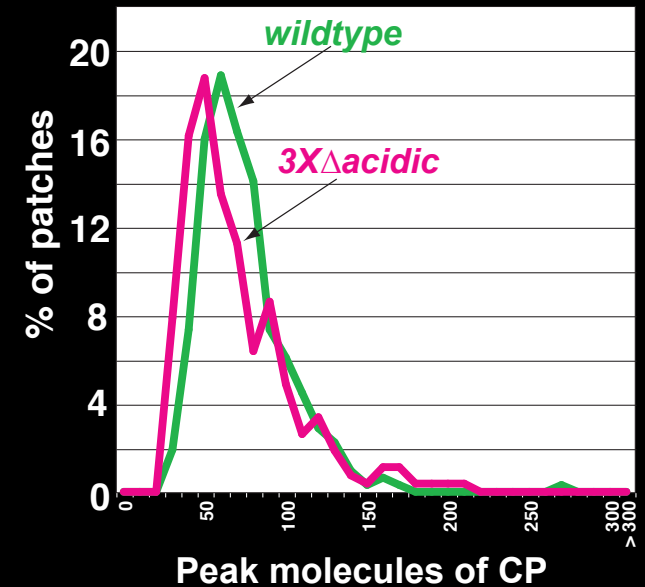
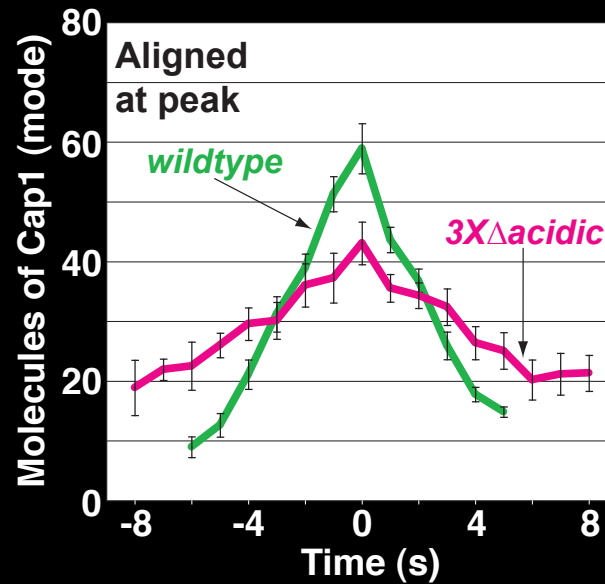
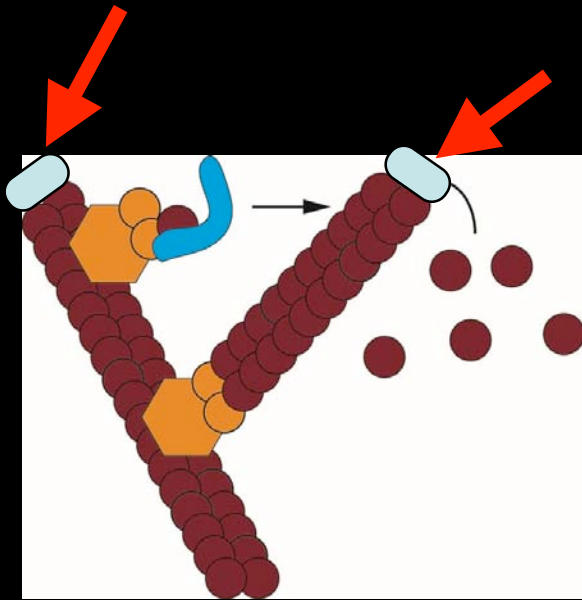


Arp2/3 levels in WASp/Las17 Myosin-I/Myo5 Double Mutant



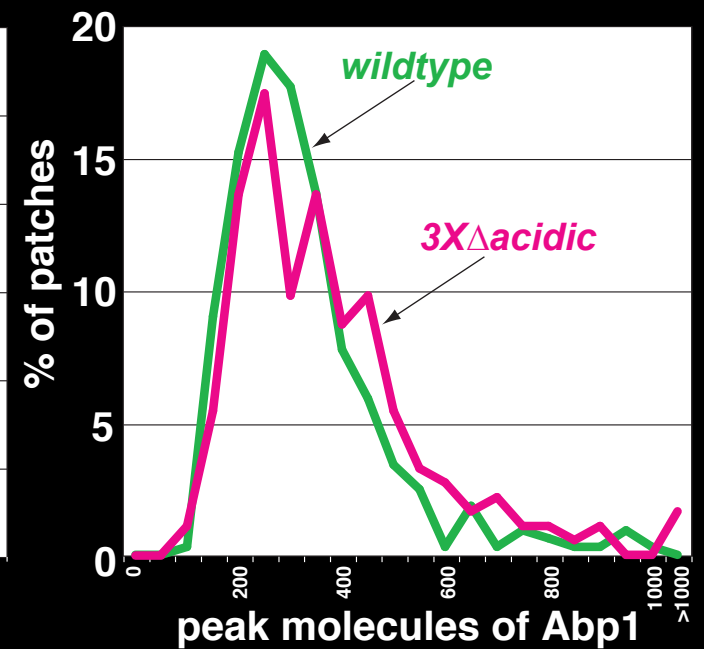
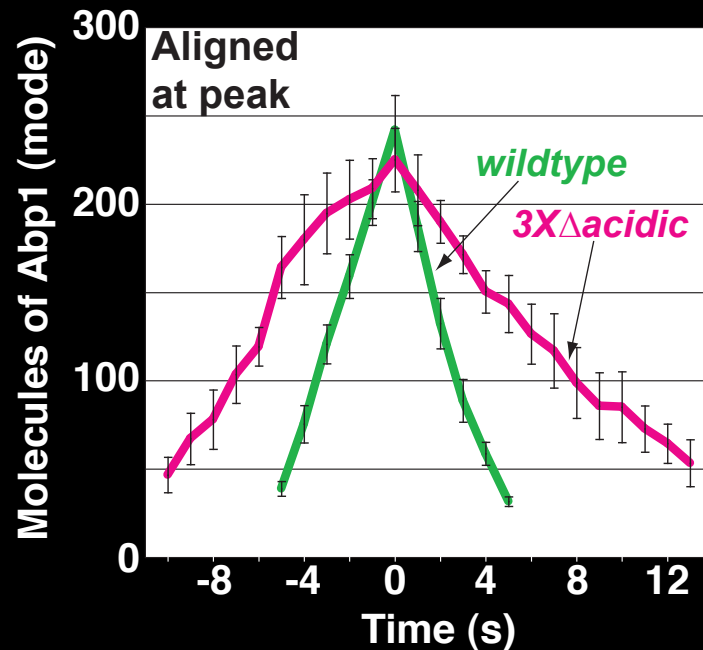
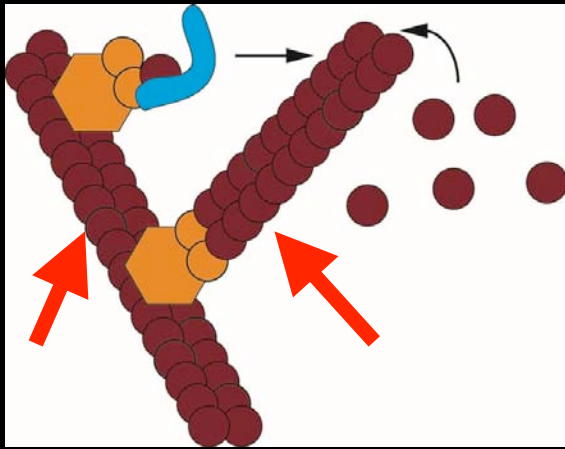
- Peak level is decreased ... but only by $\sim 1/3$
- Many patches have normal levels ... but do not move
- Rate & Duration of Assembly & Disassembly Altered

Capping Protein Levels in WASp/Las17 Myosin-I/(Myo3&5) Triple Mutant



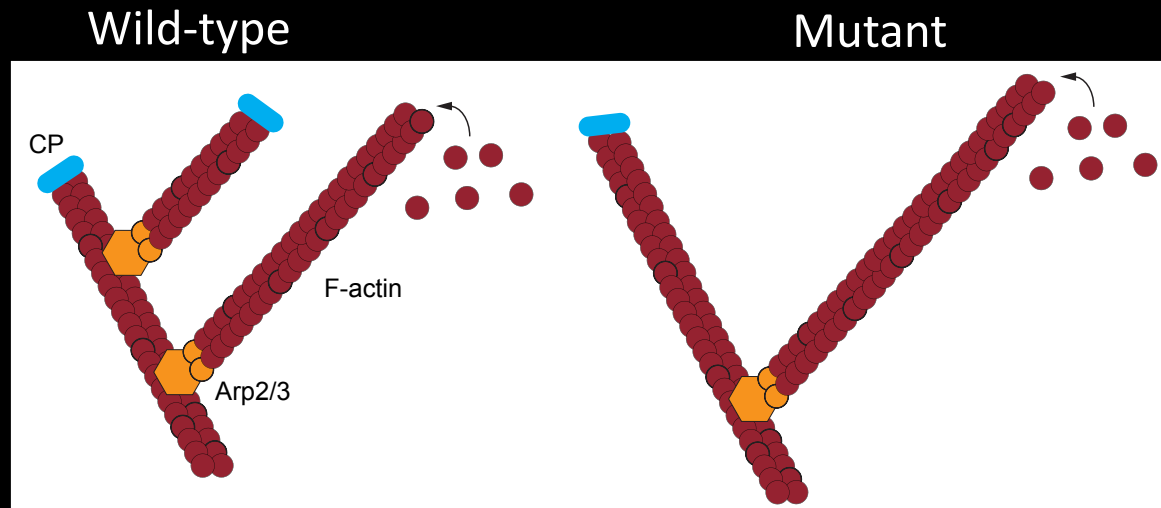
- Peak level is decreased ... but only by $\sim 1/3$
- Very many patches have normal levels ... but do not move
- Rate & Duration of Assembly & Disassembly Altered

Abp1 Levels in WASp/Las17 Myosin-I/(Myo3&5) Triple Mutant



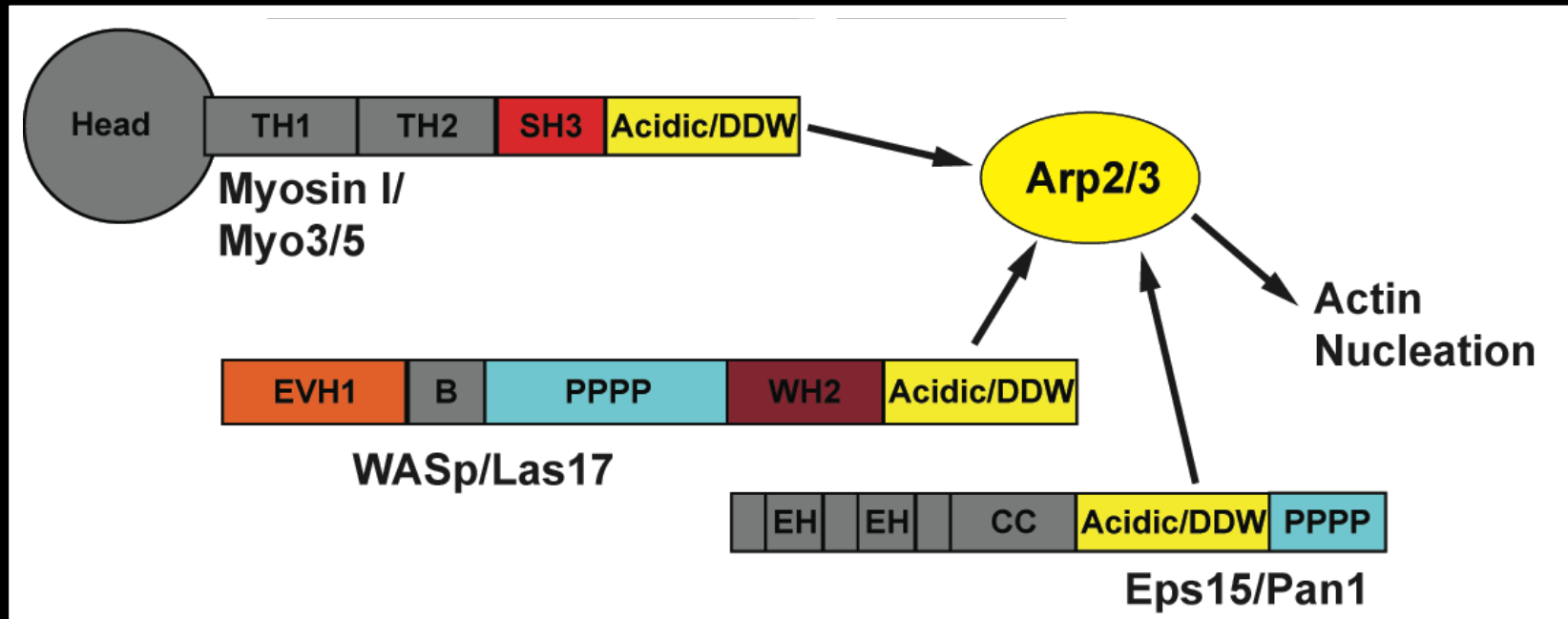
- Peak actin levels: Normal
- Rate & Duration of Assembly & Disassembly Altered

Summary for WASp/Las17 Myosin-I Mutants



- Cause of Strong Motility Defect?
 - ↓ Peak Levels of Arp2/3 and CP ... but only 1/3?
 - No Δ Peak F-actin Levels
 - ↓ Rates Assembly & Disassembly
- Defect in Architecture of Filament Network?
 - Are the Branches Fewer and Longer?

Summary for Other Mutations



Mutant (Δ acidic)

myo3 myo5 double.....

Phenotypes

No changes

pan1 single.....

No changes

myo3 myo5 pan1 triple.....

↓ peak level Arp2/3

NI peak levels CP & Actin

↓ rates of assembly and loss

las17 pan1 double.....

↑ peak levels Arp2/3, CP & actin

↑ rate of Arp2/3 & actin accumulation

Conclusions for Arp2/3 Regulator Mutants

- Motility defects appear not to be explained by...
 - Insufficient recruitment of Arp2/3
 - Insufficient activation of Arp2/3
- Are there defects in the architecture of the actin filament network?
 - Changes in rates of patch assembly and disassembly

Acknowledgments



Brian Galletta
NHLBI, NIH



Anders Carlsson
Physics, Wash U

Undergraduates

- Dennis Chuang, Wash U
- Kevin Schmidt, Wash U
- Parker Seidel, Princeton

Funding NIH / NIGMS



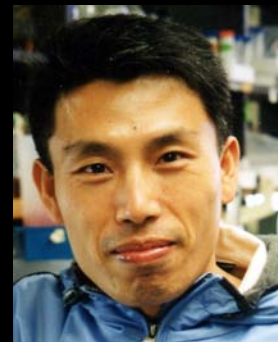
Shandiz Tehrani
Univ Oregon



Meng-Chi Lin
RIKEN Center, Kobe



Michael Young
Concordia Univ



Kyoungtae Kim
Missouri State Univ



Dorothy Schafer
Univ Virginia



Alissa Weaver
Vanderbilt