Quantitative analysis of actin-based endocytosis and cytokinesis

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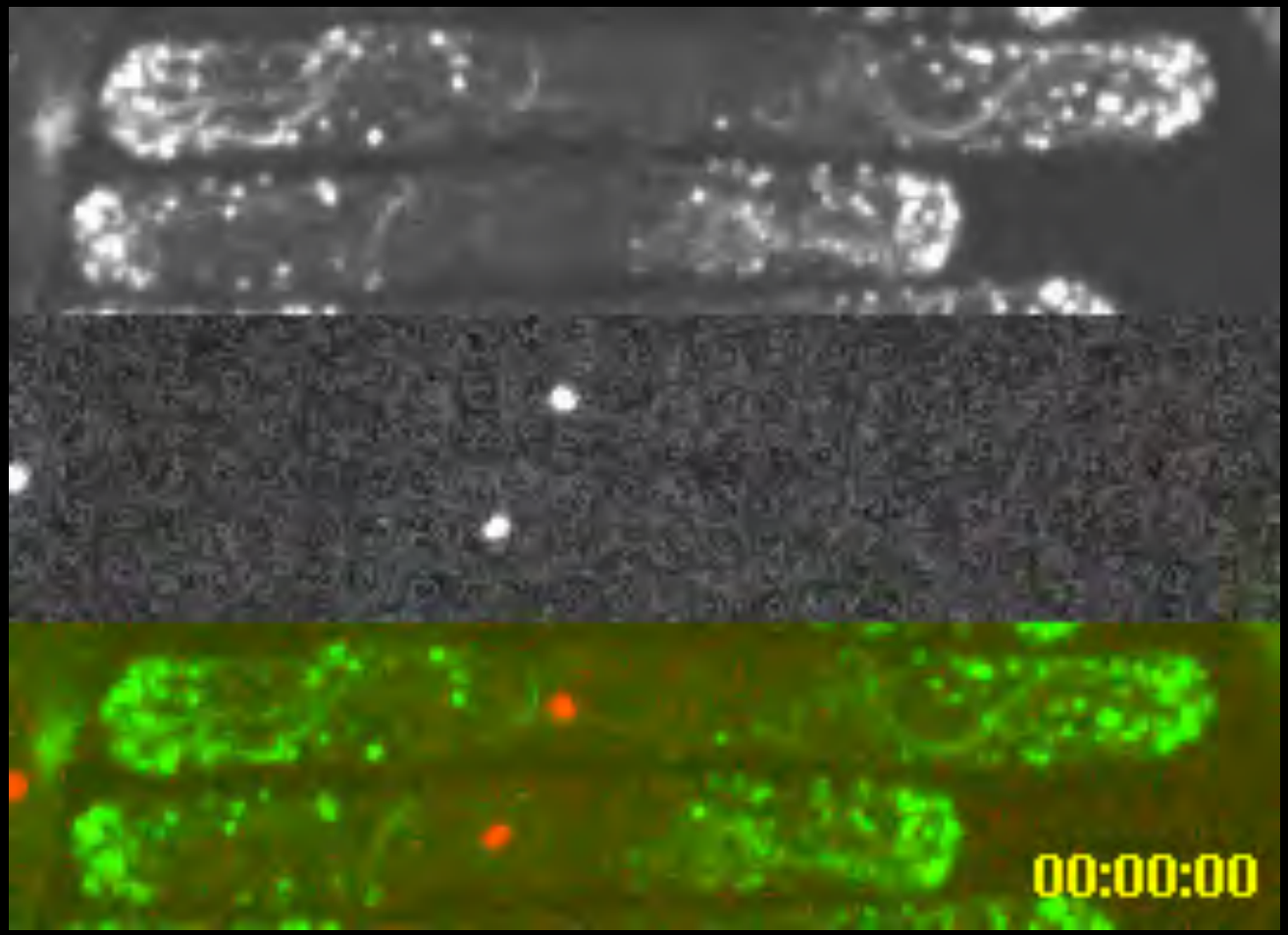
Fission yeast cytokinesis: nodes containing myosin-II and formin Cdc12p condense into a contractile ring when actin filaments assemble at time zero

GFP-CHD
actin
filaments

Sad1p-RFP
spindle pole
bodies

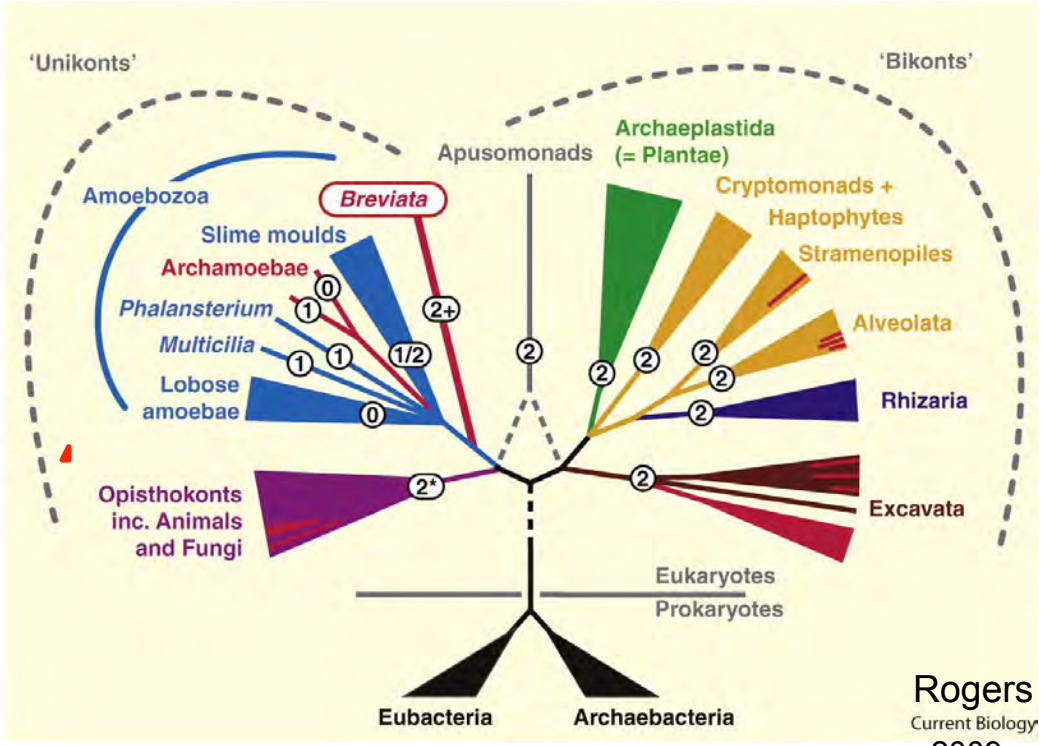


Red spindle pole bodies and green actin filaments

Jian-Qiu Wu & Dimitris Vavylonis

Why study cytokinesis & motility in fission yeast?

Phylogenetic tree 2009



Cytokinesis gene list, 2009

Furrow placement	
GIN4 family kinase	Cdr2p
S/T kinase	Kin1p
Anillin	Mid1/Dmf1p
γ-tubulin complex	Mto1/Mbo1p, Mto2p
Polo kinase	Plo1p
DYRK kinase	Pom1p
Ring assembly	
Capping protein	Acp1p, Acp2p
Actin	Act1/Cps8p
Cofilin	Adf1/Cof1p
Alpha-actinin	Ain1p
Aurora-B kinase	Ark1/Aim1p
MT cross-linking	Ase1p
Survivin	Bir1/Cut17p
Novel protein	Blt1p?
Formin	Cdc12p
F-BAR/PCH proteins	Cdc15p, Imp2p
Profilin	Cdc3p
Myosin-II LC	Cdc4p
Tropomyosin	Cdc8p
Fimbrin	Fim1p
Rho GEF	Gef2p?
Hsp90 chaperone	Hsp90/ Swo1p
Kinesin	Klp8p?
Myosin-II HC	Myo2, Myp2p
PI-dependent kinase	Pdk1p
INCENP	Pic1p
GEF for Rho1p	Rgf3/Lad1p
Myosin-II RLC	Rlc1p
IQGAP	Rng2p
UCS protein	Rng3p
Constriction and disassembly	
γ-tubulin complex subunit	Alp4p
SIN component	Cdc14p
SIN GAPs	Cdc16/Bub2p, Byr4p
SIN kinase	Cdc7/Pld1p
Cdc14 phosphatase	Clp1/Flp1p
Spindle checkpoint	Dma1p

Nucleolar protein	Dnt1p
Ethanol-dependent mutant protein	Etd1p
C2 domain protein	Fic1p
Cyclin	Lsc1p
Cdk	Lsk1p
Sid2p complex subunit	Mob1p
APC subunit	Nuc2/Apc3p
PP2A B' subunits	Par1p, Par2/Pbp2p
Paxillin	Pxl1p
14-3-3 proteins	Rad24p, Rad25p
PAK (p21-activated kinase)	Shk1/Pak1/ Orb2p
SIN kinase	Sid1p
SIN Ndr-family kinase	Sid2p
SIN scaffold proteins	Sid4p, Cdc11p
Shk1p binding protein	Skb15p
SIN GTPase	Spg1/Sid3p
Zinc-finger protein	Zfs1/Moc4p
Membrane fusion	
Transcription factor	Ace2p
Alpha-glucanase	Agn1p
α-glucan synthase	Ags1/Mok1p
Clathrin adaptor	Apm1p
Arp2/3 complex	Arp2/3
Batten-disease gene *CLN 3*	Btn1p
Chitin synthases	Chs2p, Chr4/Cfh3p
Beta-glucan synthases	Cps1/Bgs1/ Drc1/p, Bgs3p, Bgs4p
P-type ATPase	Cta4/Sev4p
endo-(1,3)-β-glucanase	Eng1p
Formin	For3p
GEFs for Cdc42p	Gef1p, Scd1/Ralp
Pig-Q, GPI enz	Gpi1p
BAR adaptor	Hob3p
Hsp9	Hsp9p/Scf1p
PI(4)P5 kinase	Its3p

Pig-n, GPI enz	Its8p
Anillin	Mid2p
Arp2/3 complex activators	Myo1p, Wsp1p
Myosin-V	Myo51p, Myo52p
PAK-related kinase	Nak1/Orb3p
Protein kinase C	Pck2/Sts6/ Pkc1p
P-inositol kinase	Pik1p
MAP kinase	Pmk1/Spm1p
MO25 family	Pmo25p
MAP phosphatase	Pmp1p
Ca^{2+}-ATPase	Pmr1p
Boi family protein	Pob1p
F-box protein	Pof6p
Calcineurin-like phosphatase	Ppb1p
PS synthase	Pps1p
PS decarboxylases	Psd1p, Psd2p, Psd3p
Syntaxin/SNARE	Psy1/Sso1p
Rho1p GAPs	Rga1p, Rga8p
Rho2p GAP	Rga2p
GEFs for Rho1p	Rgf1p, Rgf2p
Rho GTPases	Rho1p, Rho3p, Rho4p, Rho5p, Cdc42p
RNA-binding proteins	Scw1p, Nrd1/Msa2p
Exocyst subunits	Sec6p, Sec8p, Sec10p, Exo70p
SNAP-25/t-SNARE	Sec9p
Forkhead TF	Sep1p
Heat-repeat protein	Sip1p
GSK-3	Skp1p
Proline-tRNA	Spl1
Septins	Spn1-4p
Sec14p/PITP/ Nir2	Spo20/Sec14p
SM22/transgelin	Stg1p
Synaptobrevin/ SNAP receptor	Syb1/Snc1p
Rab11 GTPase	Ypt3p

Total >130

Pollard & Wu Nature Rev 2009

Genetics: extensive inventory of genes & mutants
Homologous recombination: ease of manipulations
Quantitative microscopy: data to test mechanisms
Biochemistry/biophysics: data to test mechanisms
Mathematical modeling: rigor in testing hypotheses

Mechanisms & general principles

Time, min

-60 Mid1p exits nucleus

-10 Broad band forms

0 SPBs separate

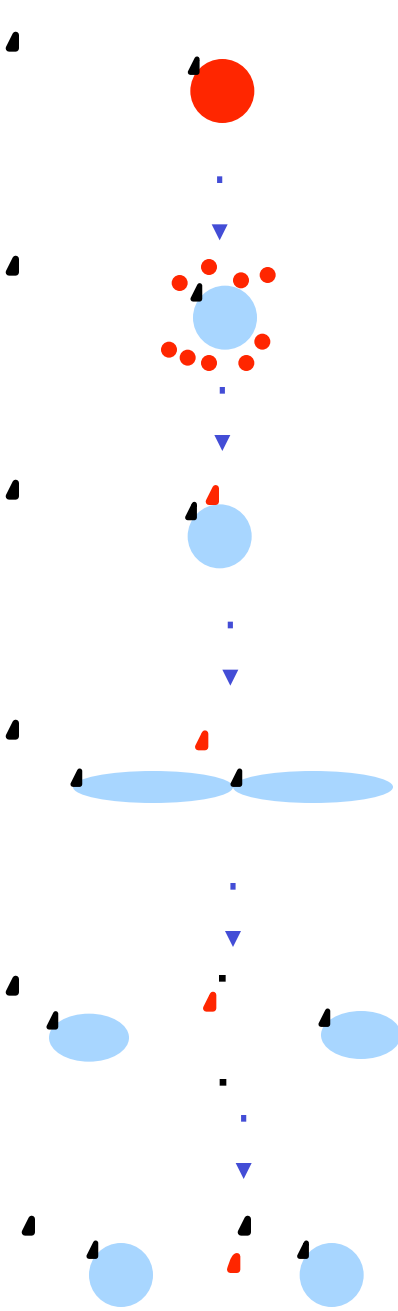
+5 Anaphase A

+10 Anaphase B

+30 End Anaphase B

+40 Constriction begins

+60 Constriction ends



Mid1p (anillin)

Myo2p (myosin-II), Rlc1p/Cdc4p

Rng2p (IQGAP)

Cdc12p (formin)

Cdc15p (PCH)

Cdc3p (profilin)

Actin filaments

Cdc8p (TM)

Fim1p (fimbrin)

Ain1p (α-actinin)

Acp1/2p (capping protein)

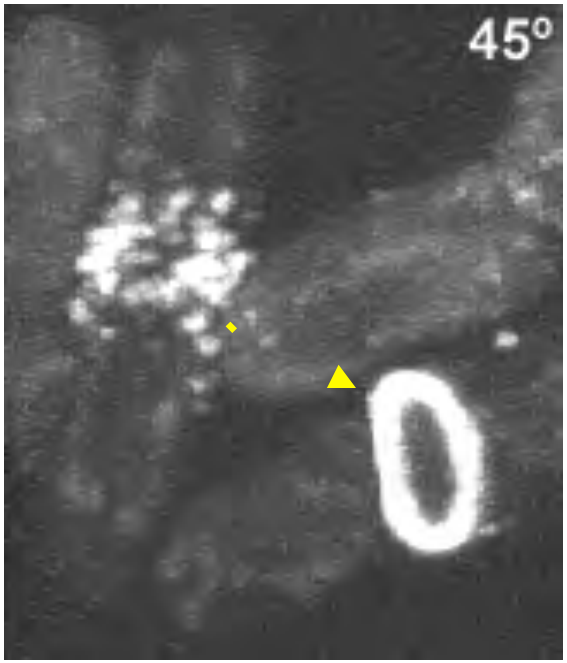
Myp2p (myosin-II)

Septins

Rng3 (UCS)

SIN pathway

Jian-Qiu Wu
Devel Cell 2003

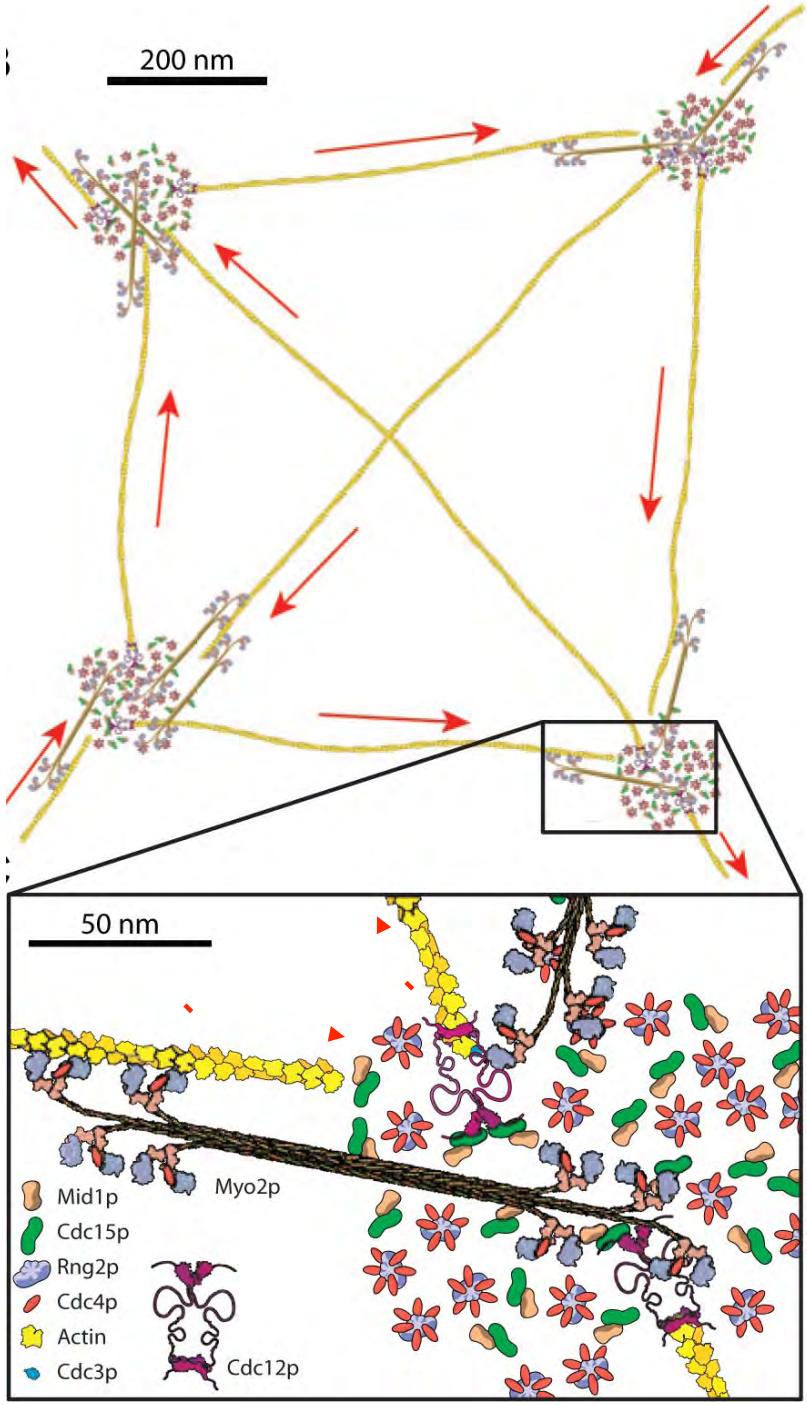


Molecules per node

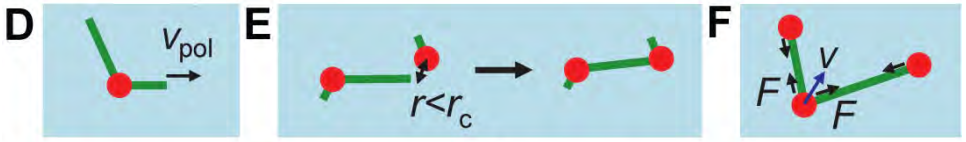
Mid1p anillin	21
Myo2 myosin-II dimers	22
Rng2p IQGAP	23
Cdc12p formin dimers	2

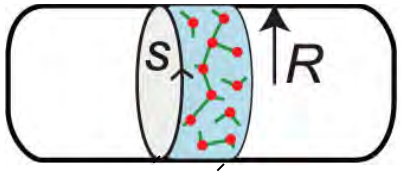
Wu et al, Science 2005

Search, capture & pull hypothesis for the transition of nodes into a contractile ring (Wu 2006):

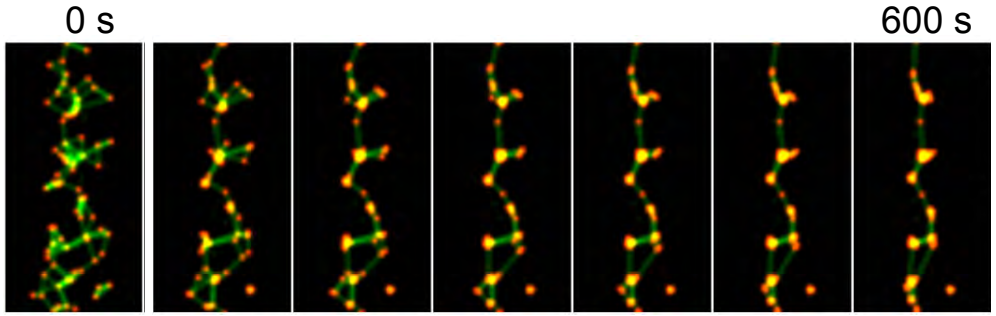


Monte Carlo simulation of simple search, capture and pull mechanism

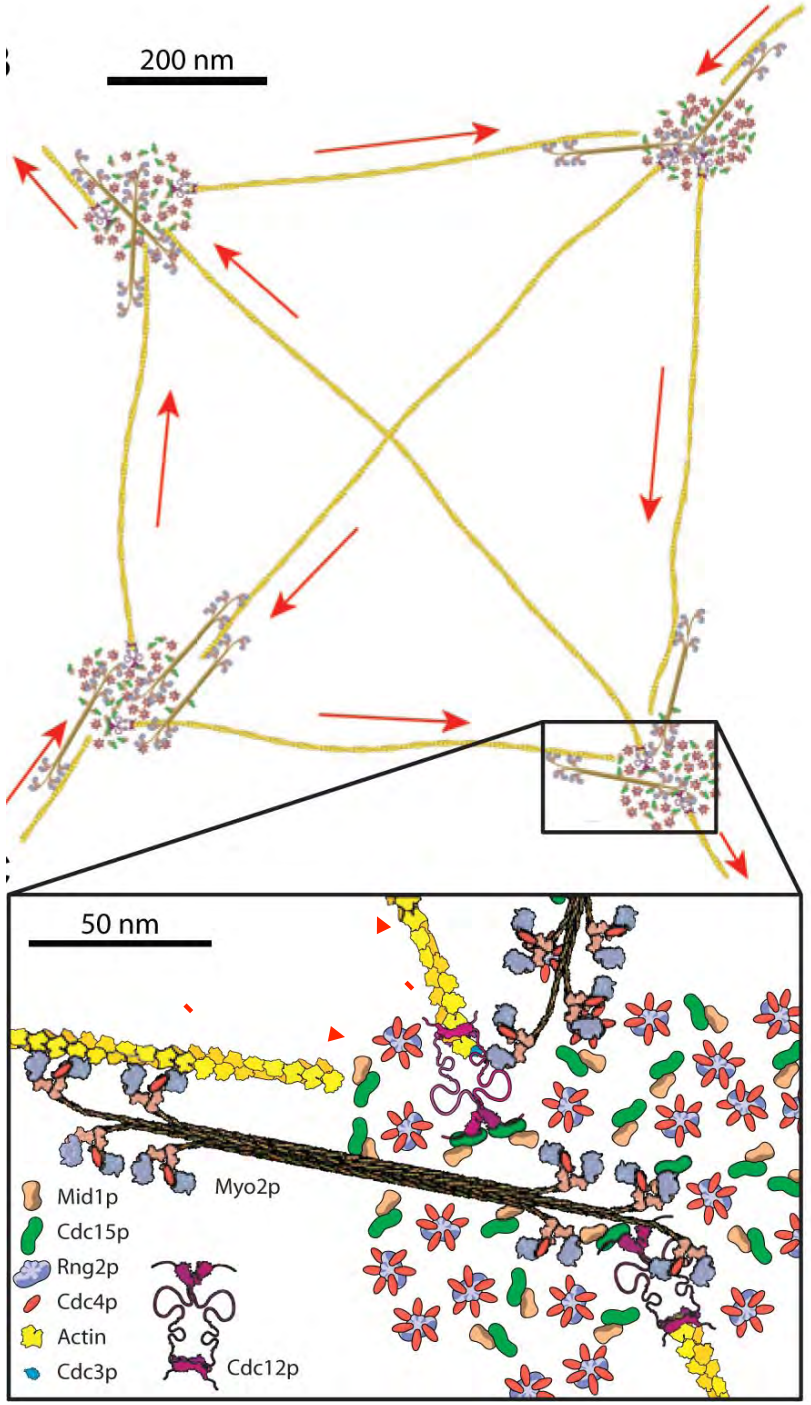




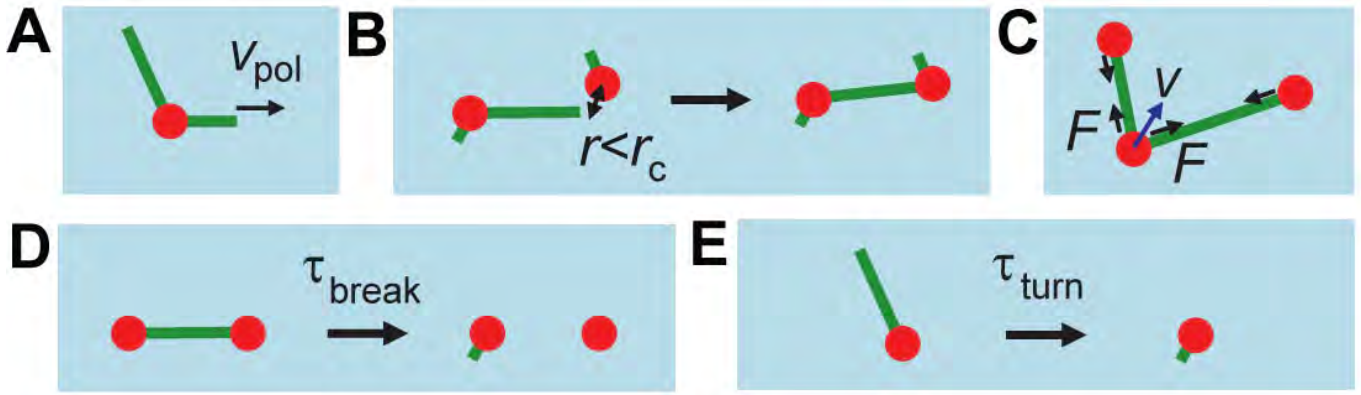
Result: ugly clumps of nodes



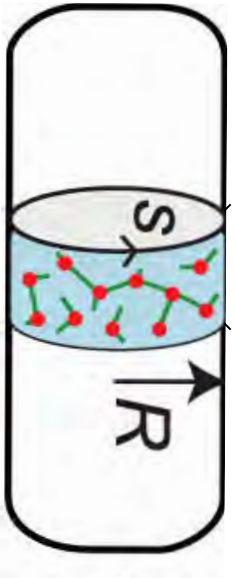
Dimitrios Vavylonis

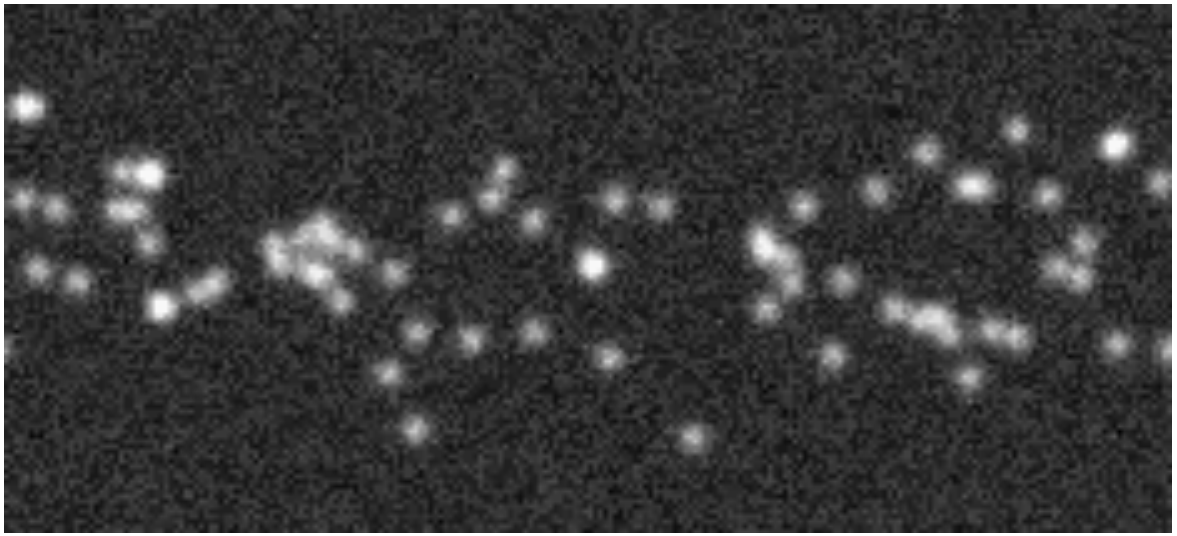


Search, capture, pull and release model for condensation of nodes into contractile rings



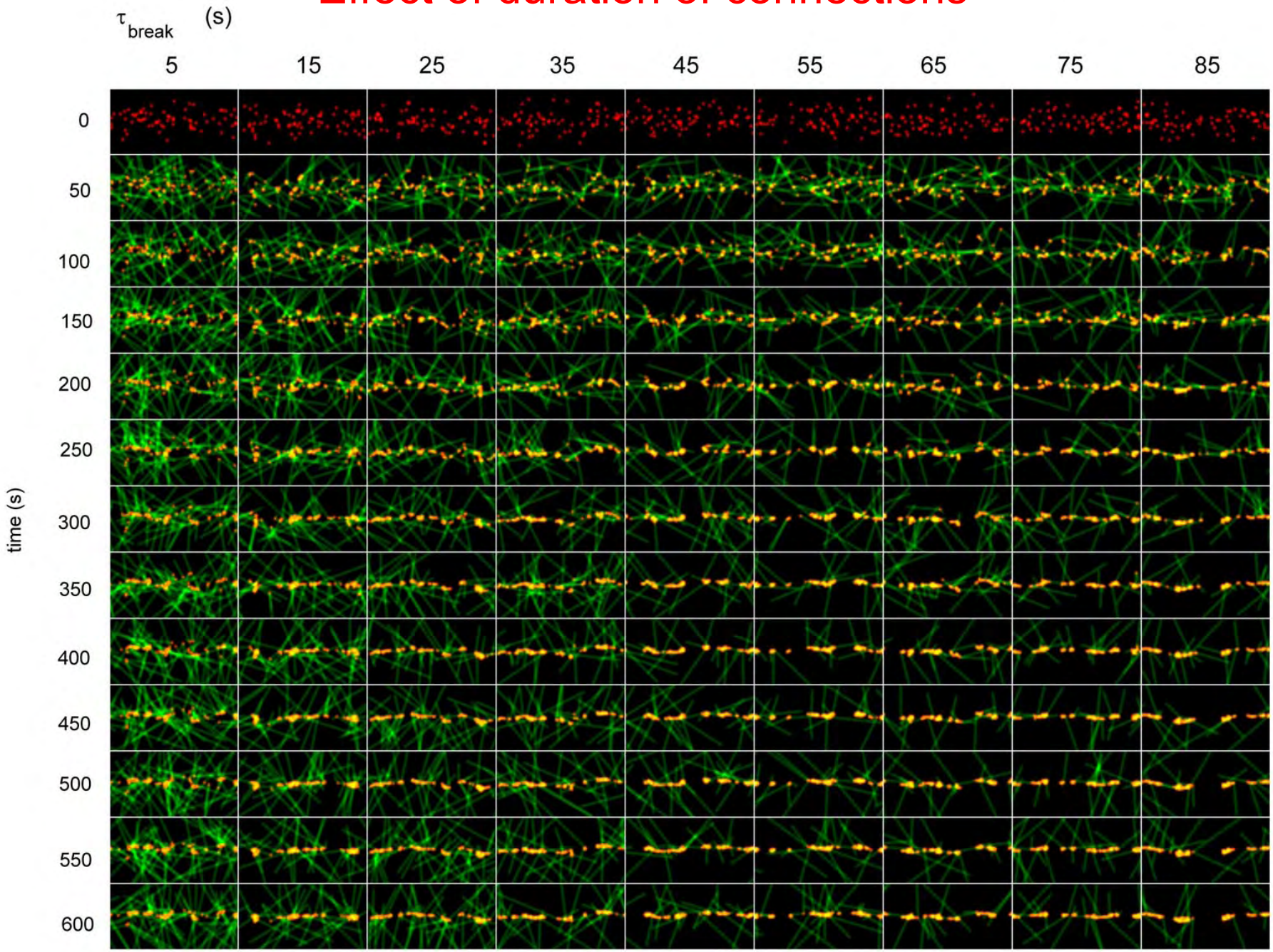
Monte Carlo simulation



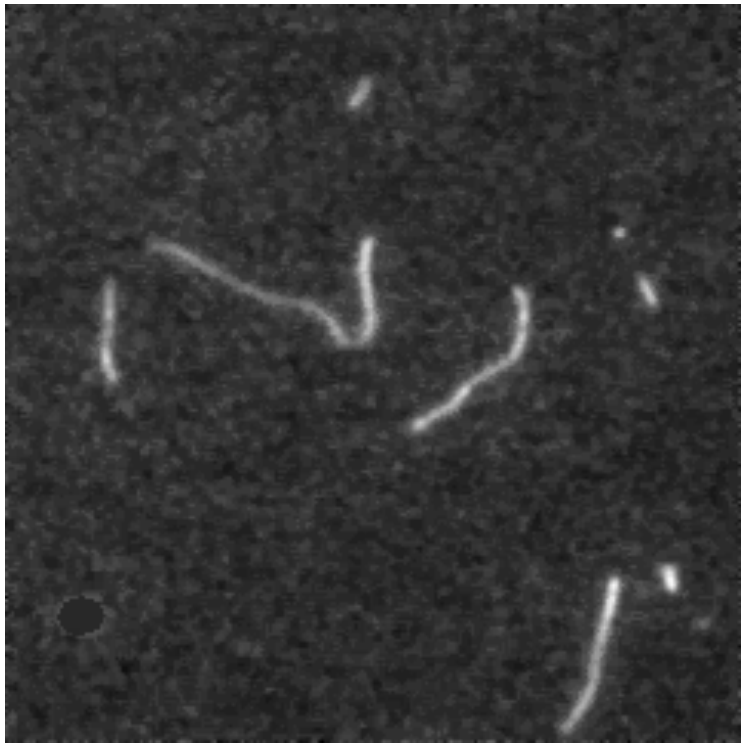


Vavylonis, Wu et al, Science 2008

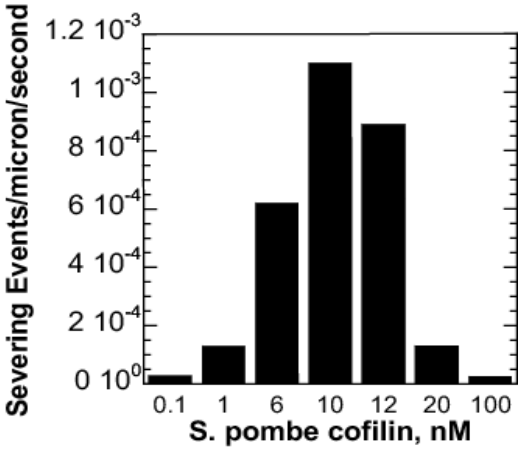
Effect of duration of connections

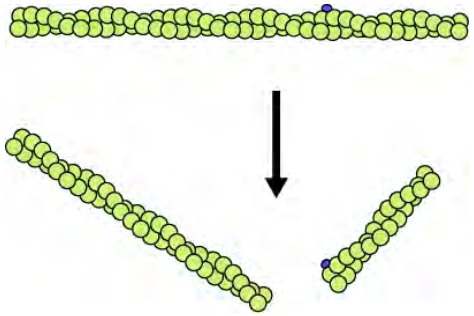


Single cofilin molecules sever ADP-actin filaments



72x time lapse



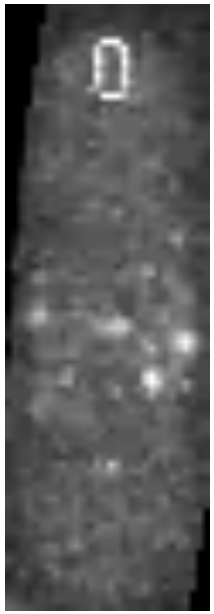


Cofilin has no effect on dissociation at pointed ends & slows dissociation at barbed ends

Andrianantoandro
Molec Cell 2007

Contractile ring assembly and stability in temperature sensitive adf-1 cells from Nakano and Mabuchi

Wild type
36°C
Rlc1p-3GFp

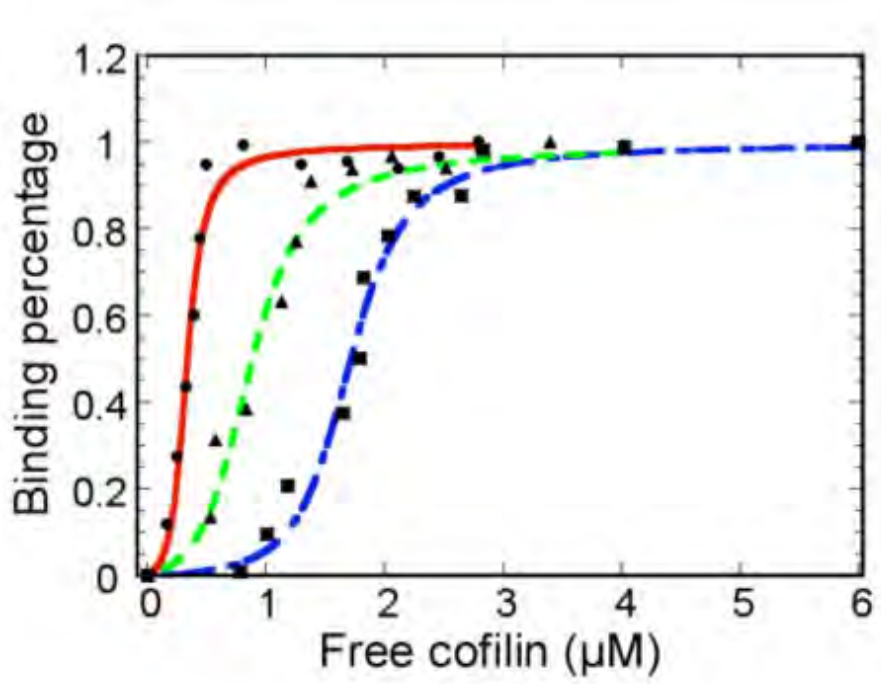


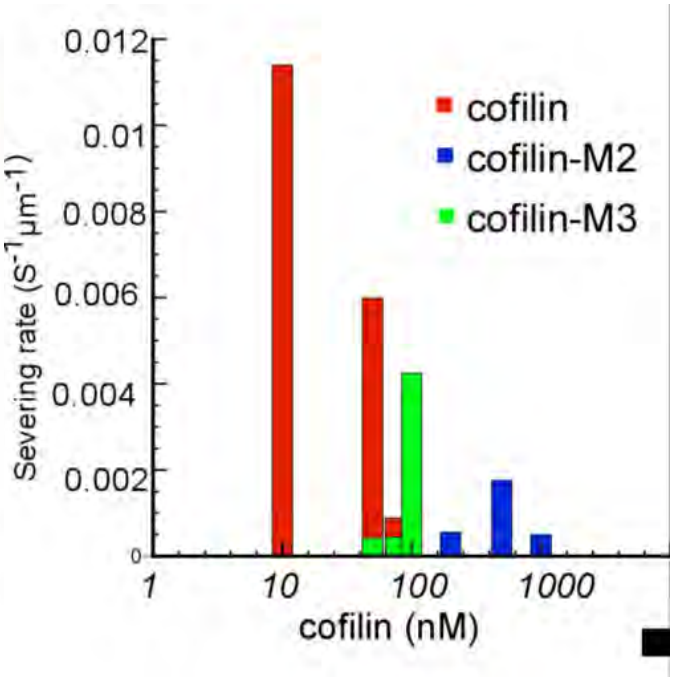
adf1-1 cell
36°C
Rlc1p-3GFp

adf1-1 cell with
contractile ring
shifted to 36°C
at movie time zero
Rlc1p-3GFp

Qian Chen, 2011

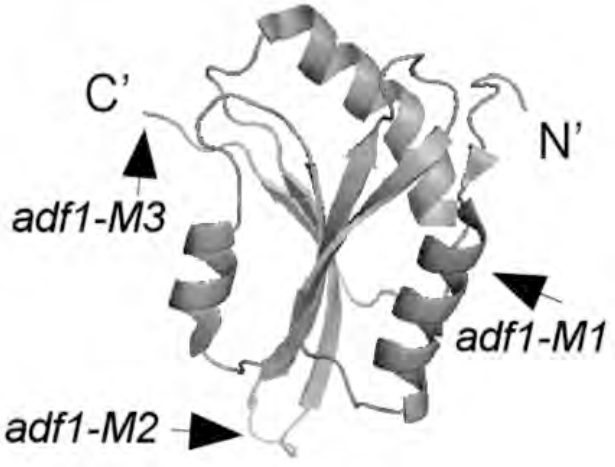
Low affinity, slowly severing cofilin mutants





E132A
K133A

K23A
K24A
K26A

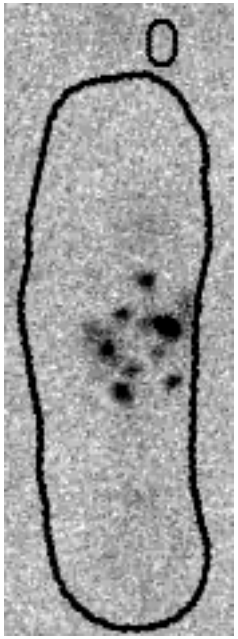


Monomer binding	normal
Filament binding	slow
Severing	slow

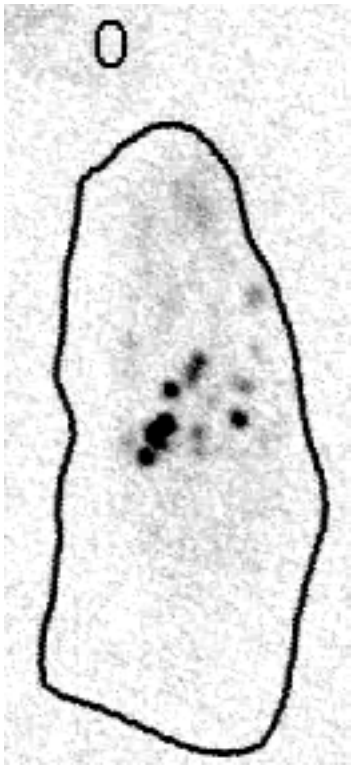
Qian Chen

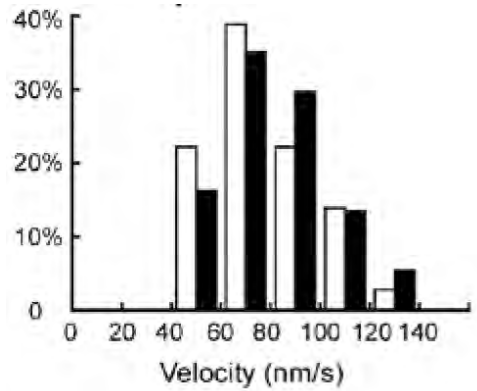
Contractile ring assembly in cells depending on slowly severing cofilin mutants

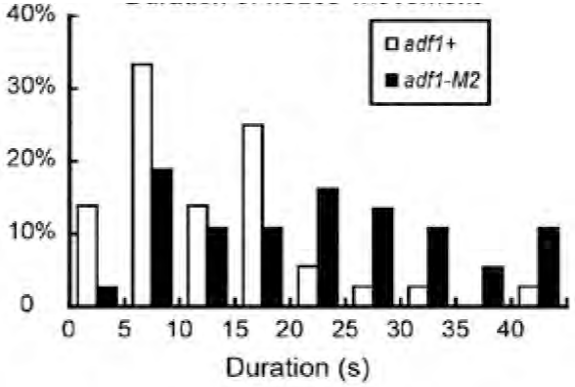
Wild type
25°C
Rlc1p-3GFp



adf1-1 cell
25°C
Rlc1p-3GFp







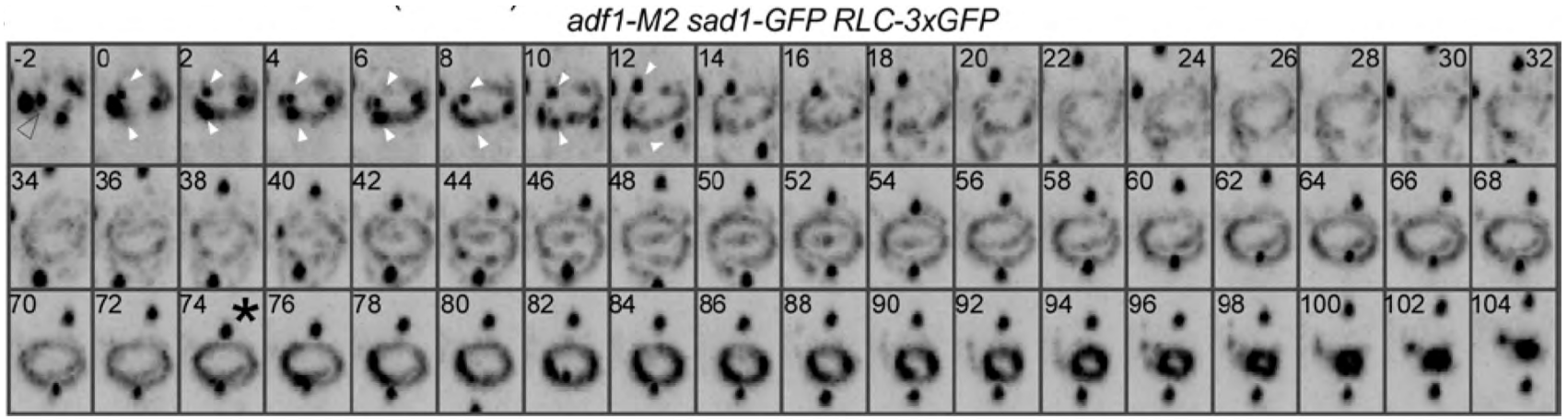
Nodes aggregate into clumps rather than coalescing into a contractile ring

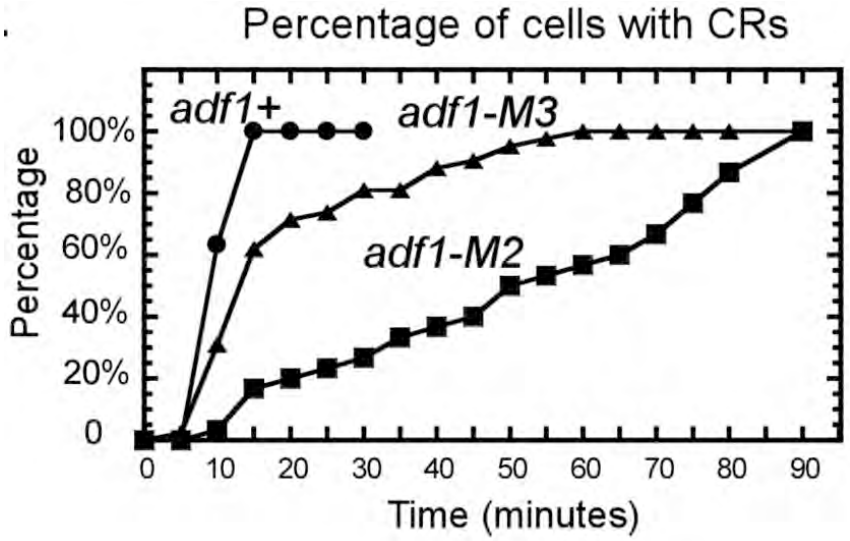
Node velocity unchanged but duration increased

Qian Chen, 2011

Low affinity, slowly severing cofilin mutants

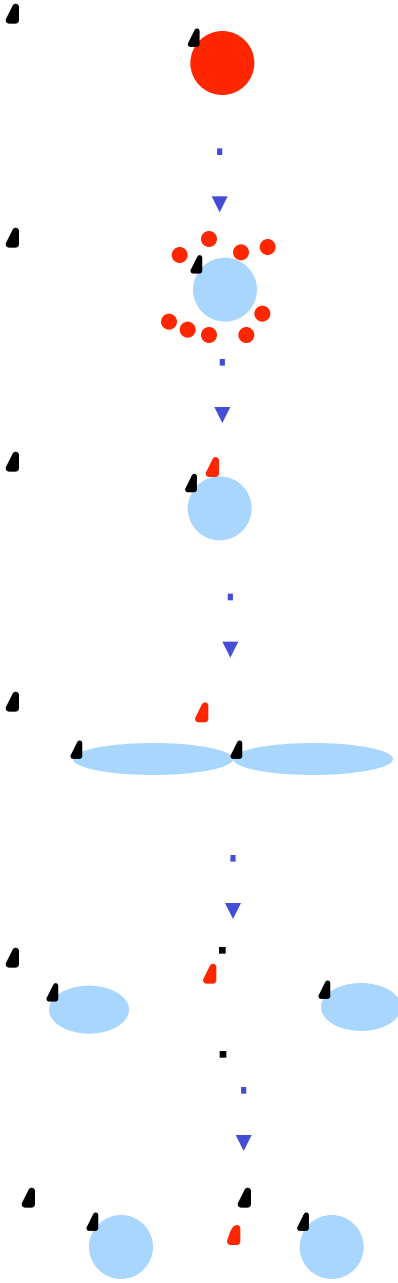
Rescue process: clumps of nodes initiate strands of actomyosin that slowly and unreliably form complete contractile rings





- Rescue requires α-actinin, which is not normally required for cytokinesis
- Mean constriction rates ~normal, but more variable

Qian Chen


Time, min
-60 Mid1p exits nucleus
-10 Broad band forms
0 SPBs separate
+5 Anaphase A
+10 Anaphase B
+30 End Anaphase B
+40 Constriction begins
+60 Constriction ends
Mid1p (anillin)
Myo2p (myosin-II), Rlc1p/Cdc4p
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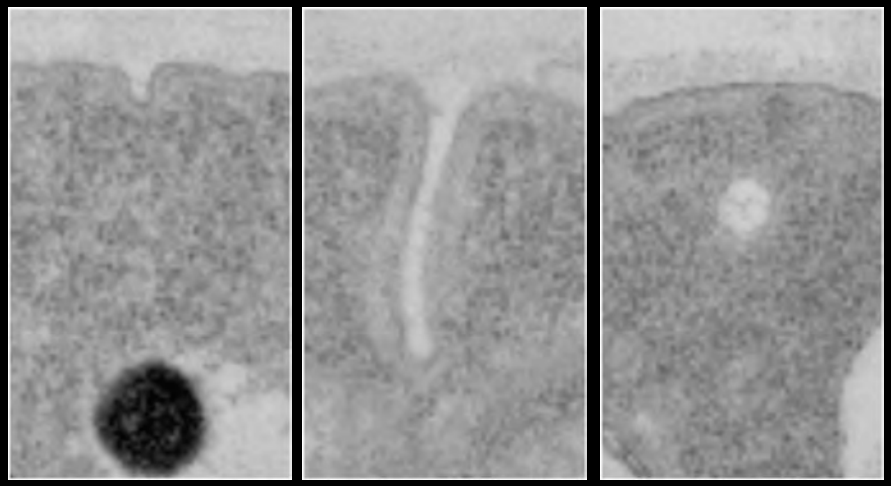
Cdc15p (PCH)
Cdc3p (profilin)
Actin filaments
Cdc8p (TM)
Fim1p (fimbrin)
Ain1p (α-actinin)
Acp1/2p (capping protein)
Myp2p (myosin-II)
Septins
Rng3 (UCS)
SIN pathway

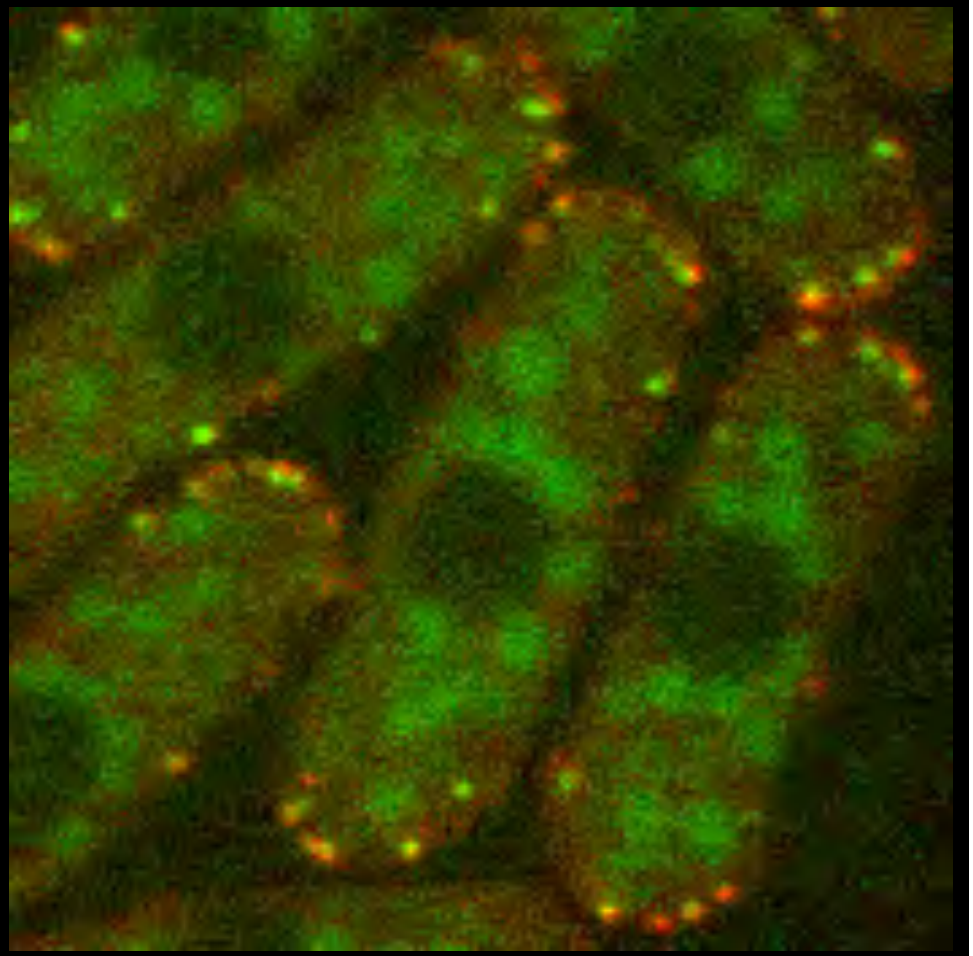
Fission yeast actin patches: model for motility



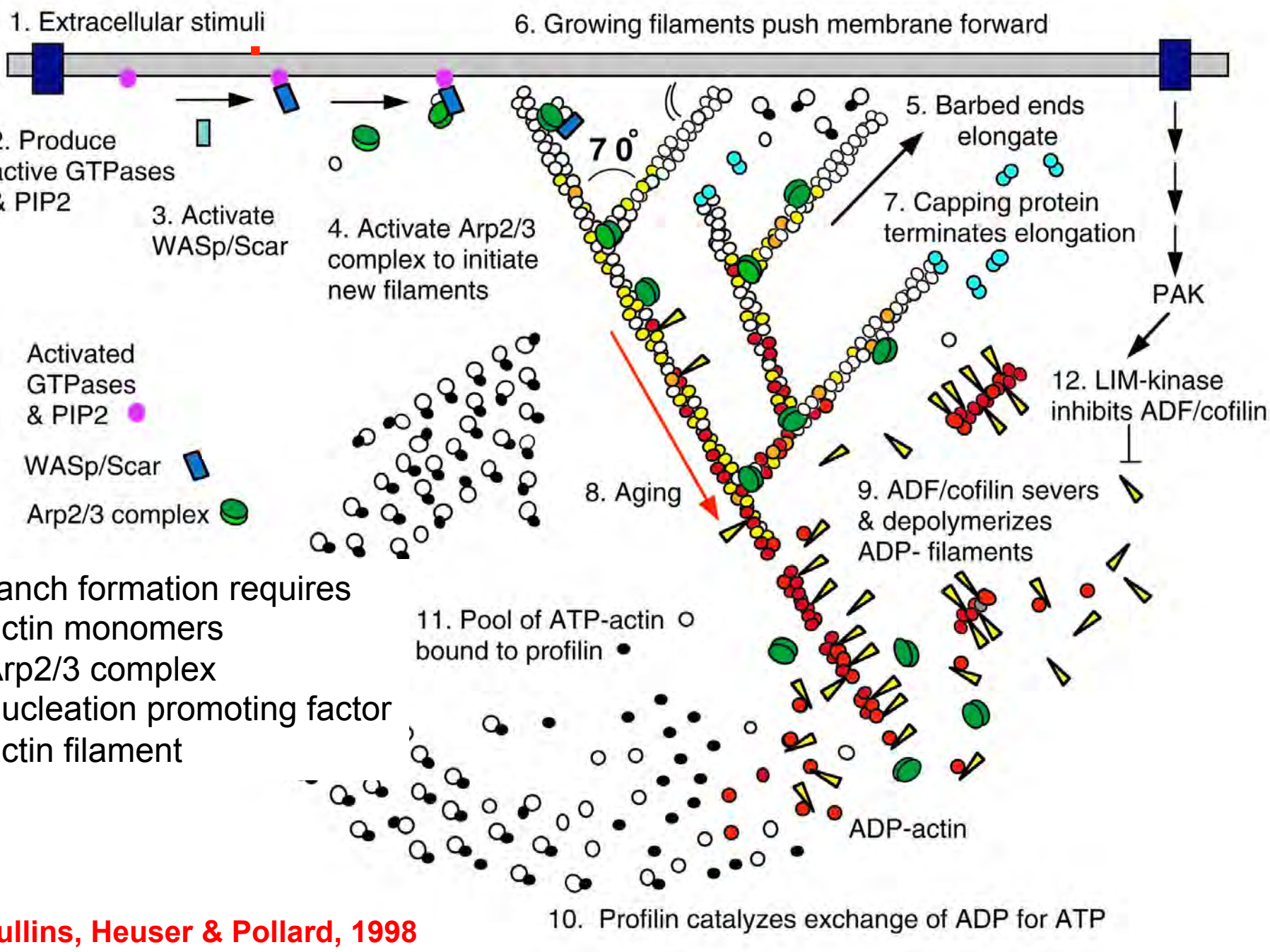
DeCamilli Lab

Volodia Sirotkin

Depends on same proteins used for protrusion of the leading edge of motile cells



Green-p16 Arp2/3 complex
Red-Wsp1p

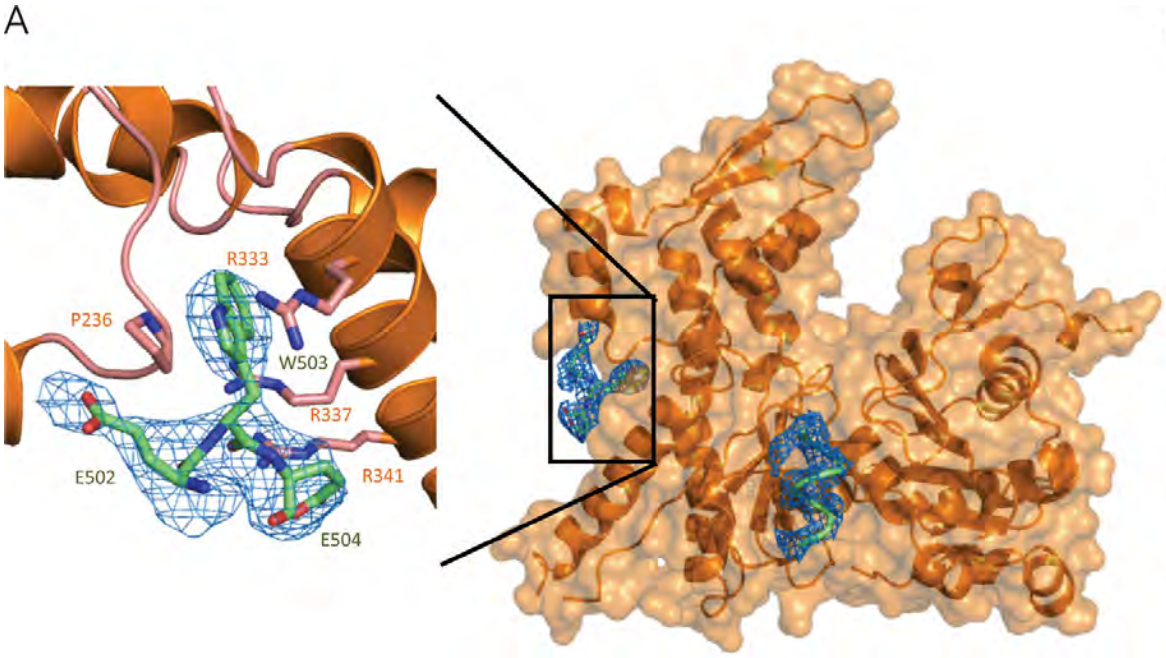


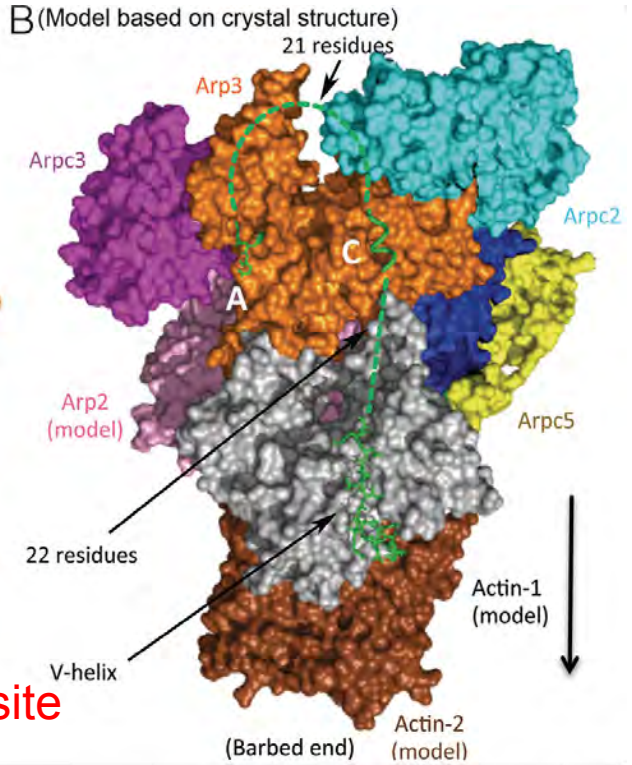
Branch formation requires

- actin monomers
- Arp2/3 complex
- nucleation promoting factor
- actin filament

Mullins, Heuser & Pollard, 1998

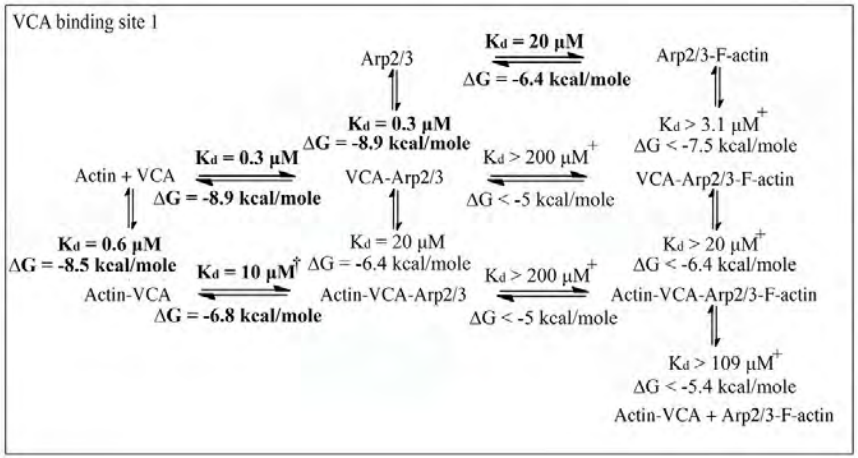
Two WASp VCA binding sites on Arp2/3 complex





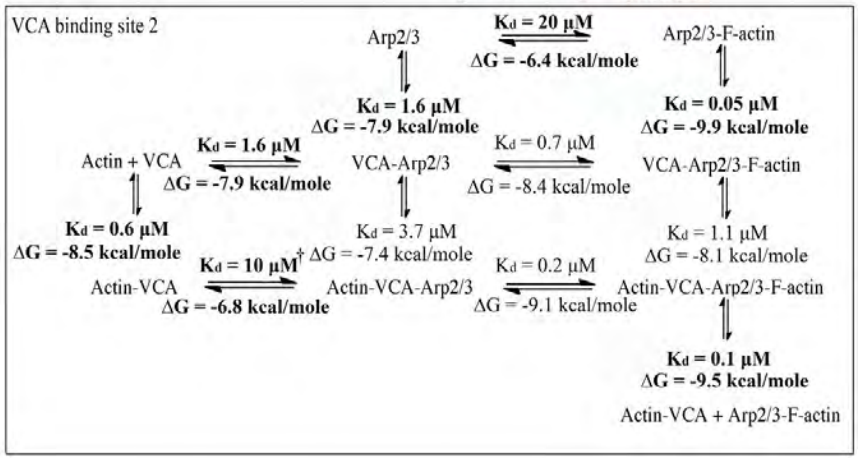
High affinity inhibitory site

C



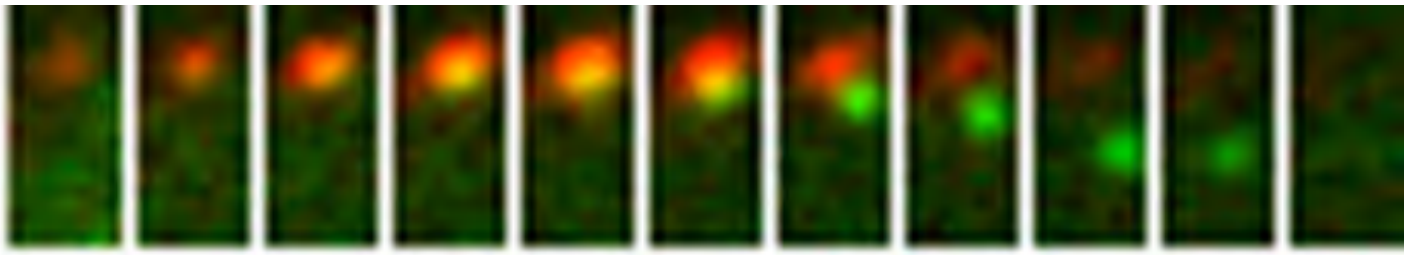
Activating site

D



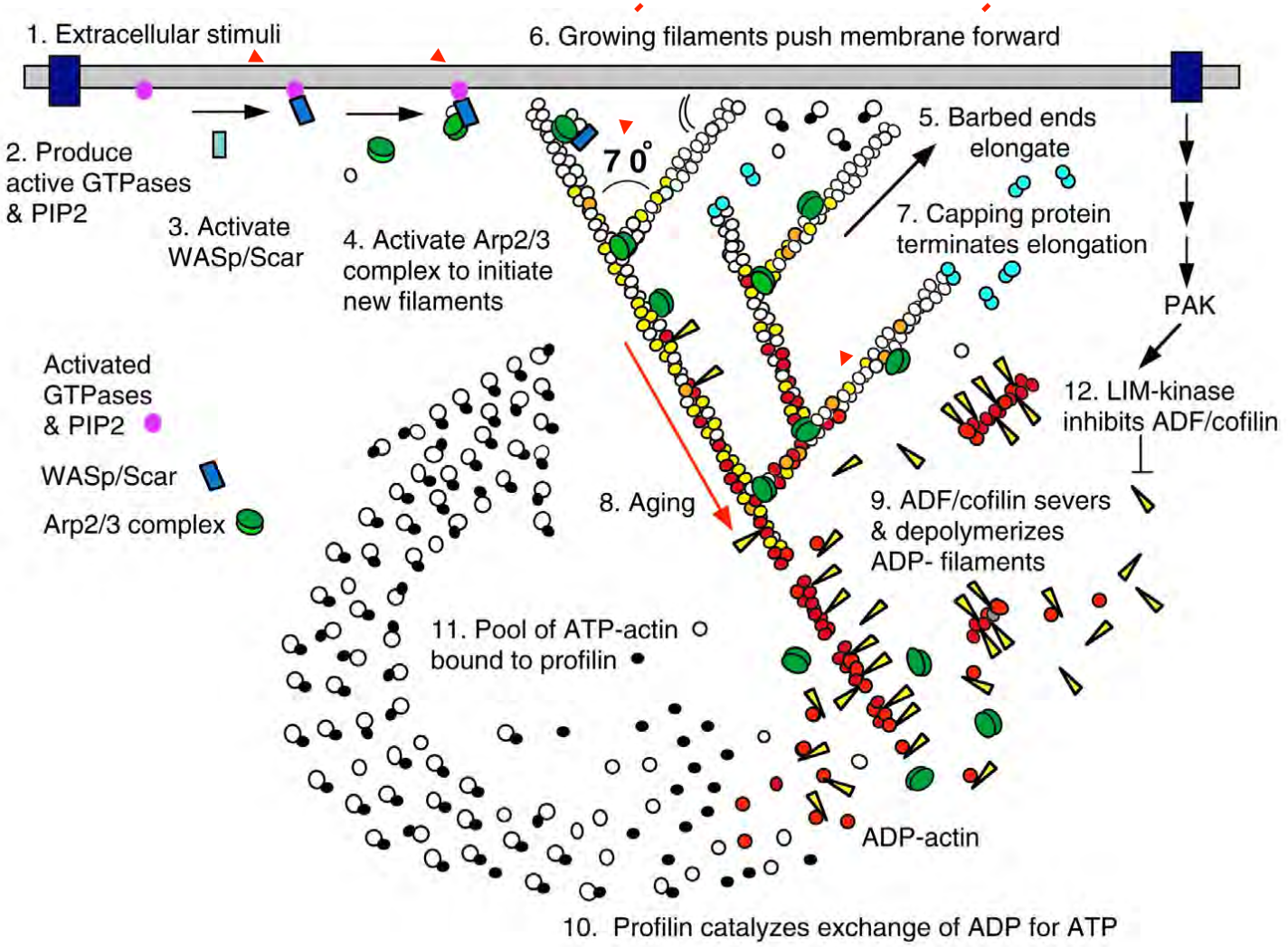
Ti, Jurgenson, Nolen & Pollard, PNAS June 2011

Green-p16
Arp2/3 complex
Red-Myo1p

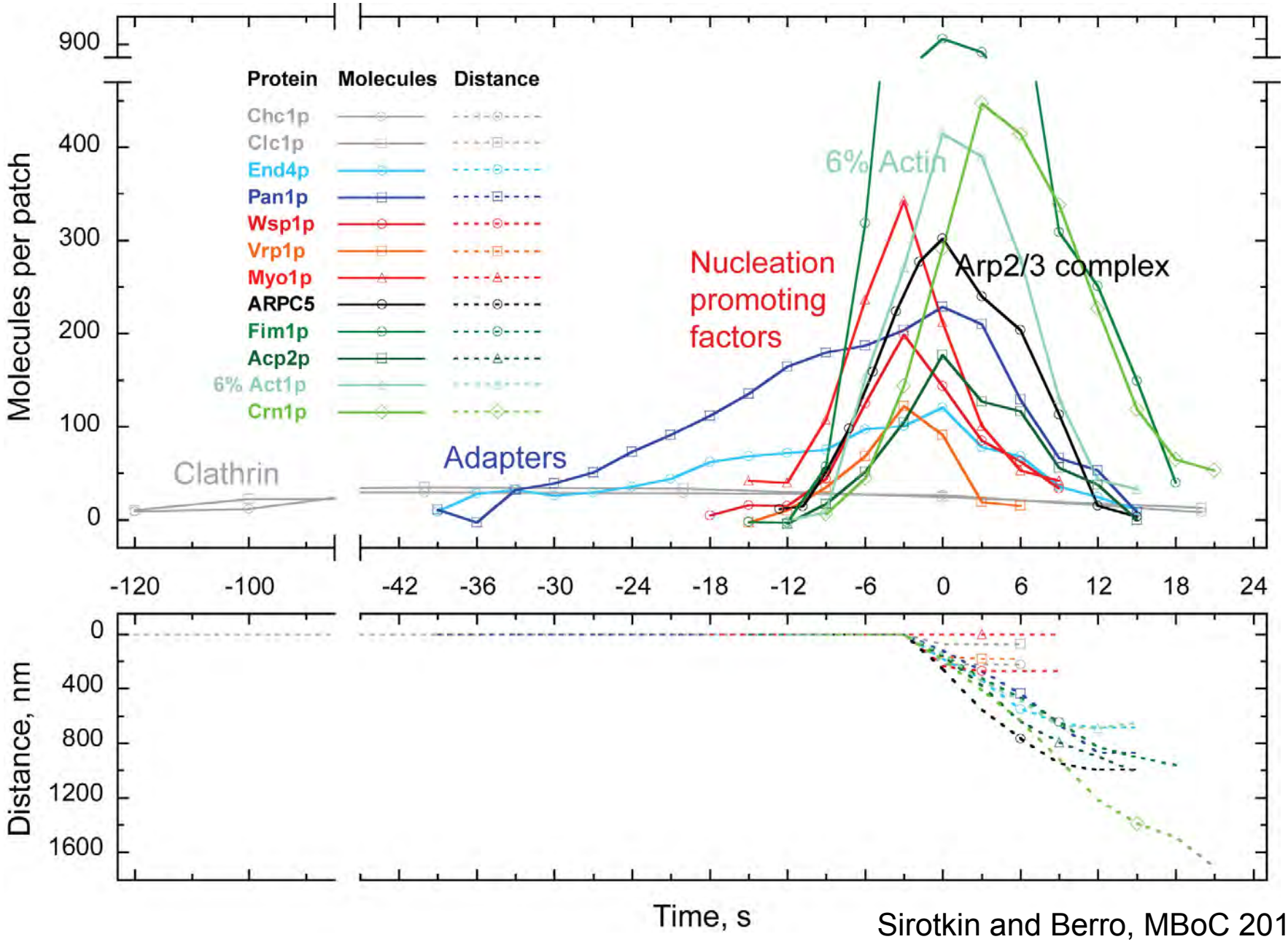


Correlation of yeast actin patch dynamics with the dendritic nucleation hypothesis:

Nucleation promoting factors: Wsp1p moves with patch but Myo1p remains on the plasma membrane

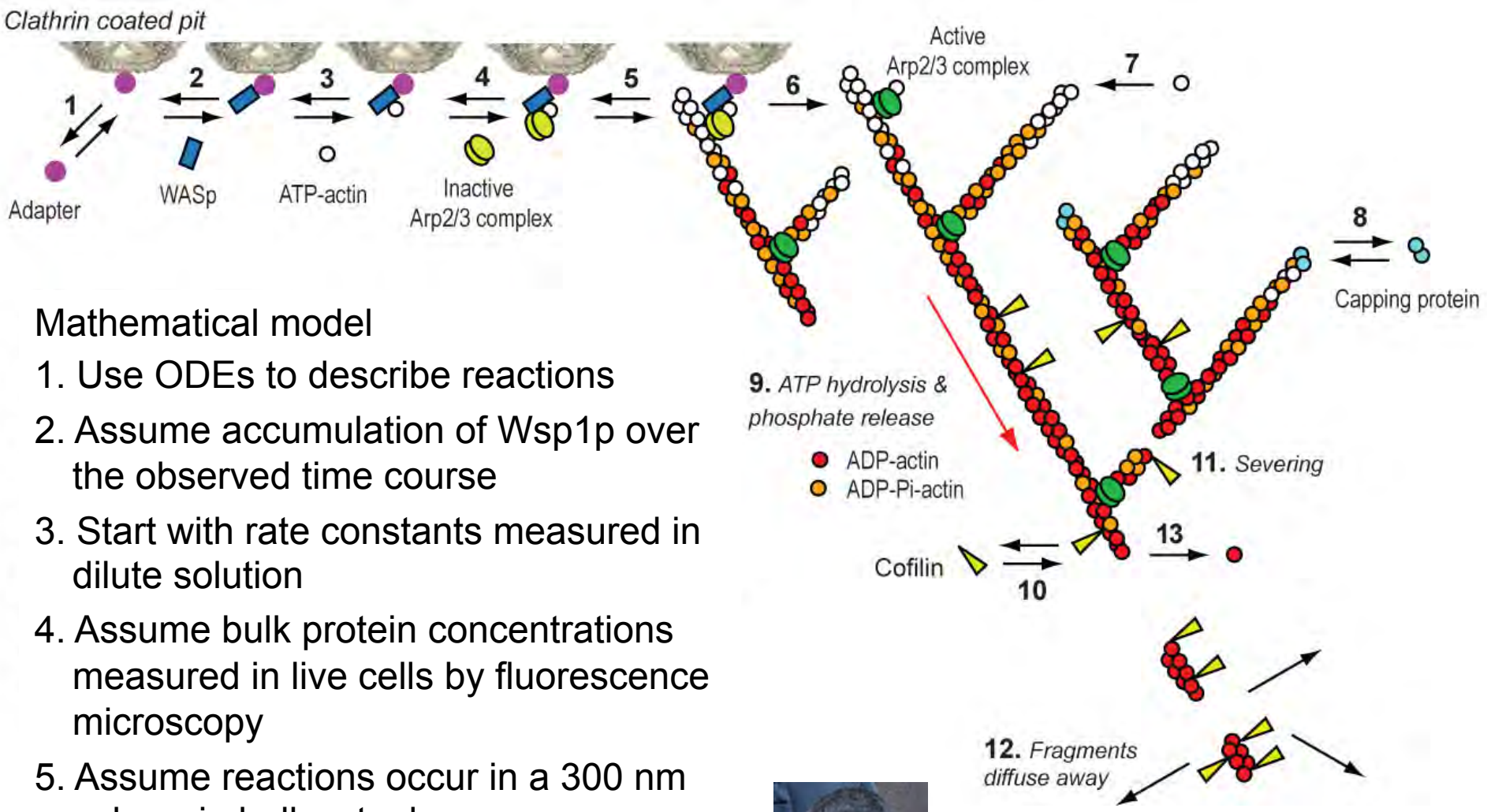


Actin patch protein composition over time



Sirotkin and Berro, MBoC 2010

Hypothesis for assembly & turnover of actin patches

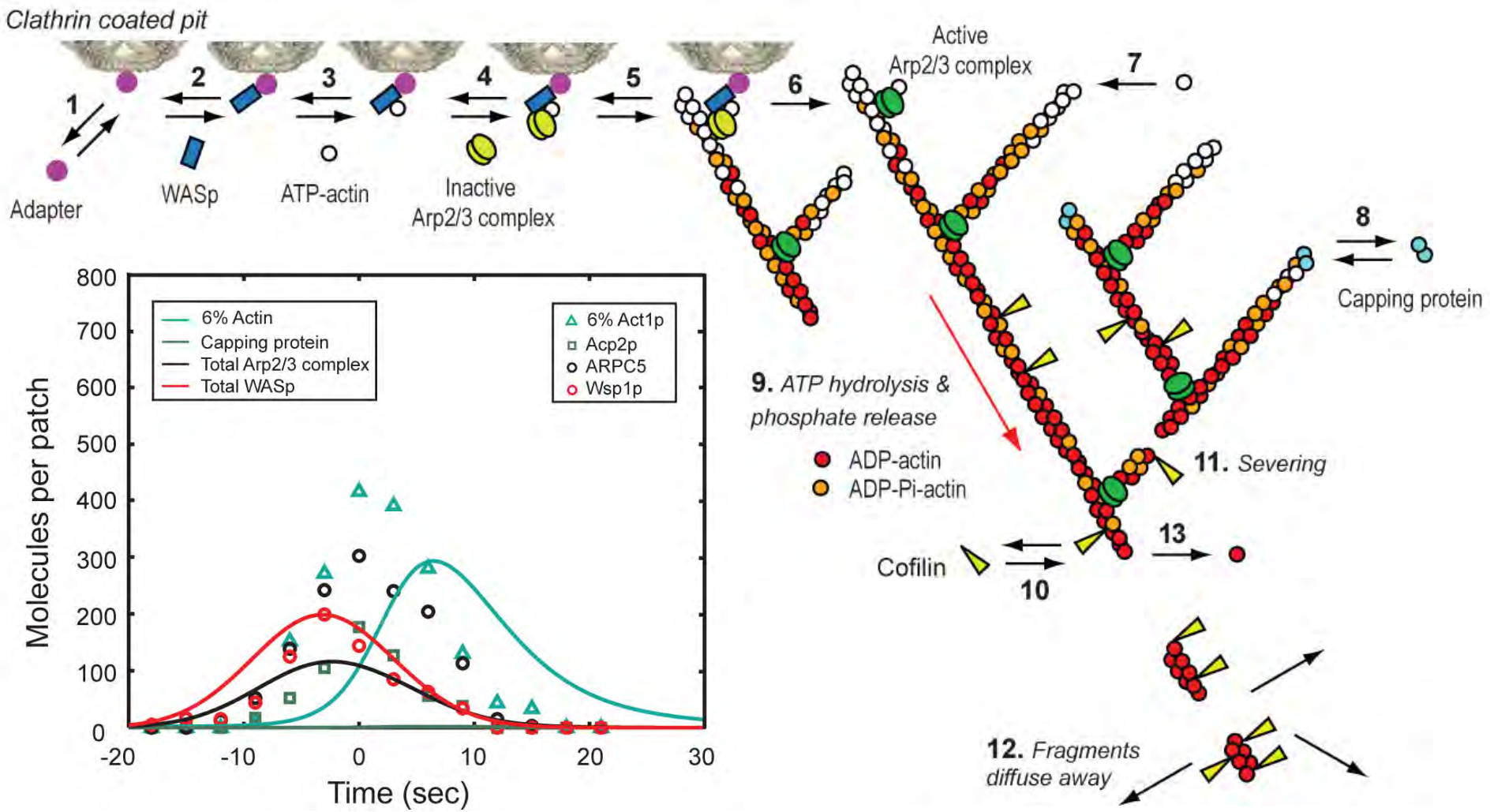


Mathematical model

1. Use ODEs to describe reactions
2. Assume accumulation of Wsp1p over the observed time course
3. Start with rate constants measured in dilute solution
4. Assume bulk protein concentrations measured in live cells by fluorescence microscopy
5. Assume reactions occur in a 300 nm sphere in bulk cytoplasm
6. Run simulations to calculate the numbers of molecules over time

Berro & Sirotkin, MBoC 2010

Hypothesis for assembly & turnover of actin patches

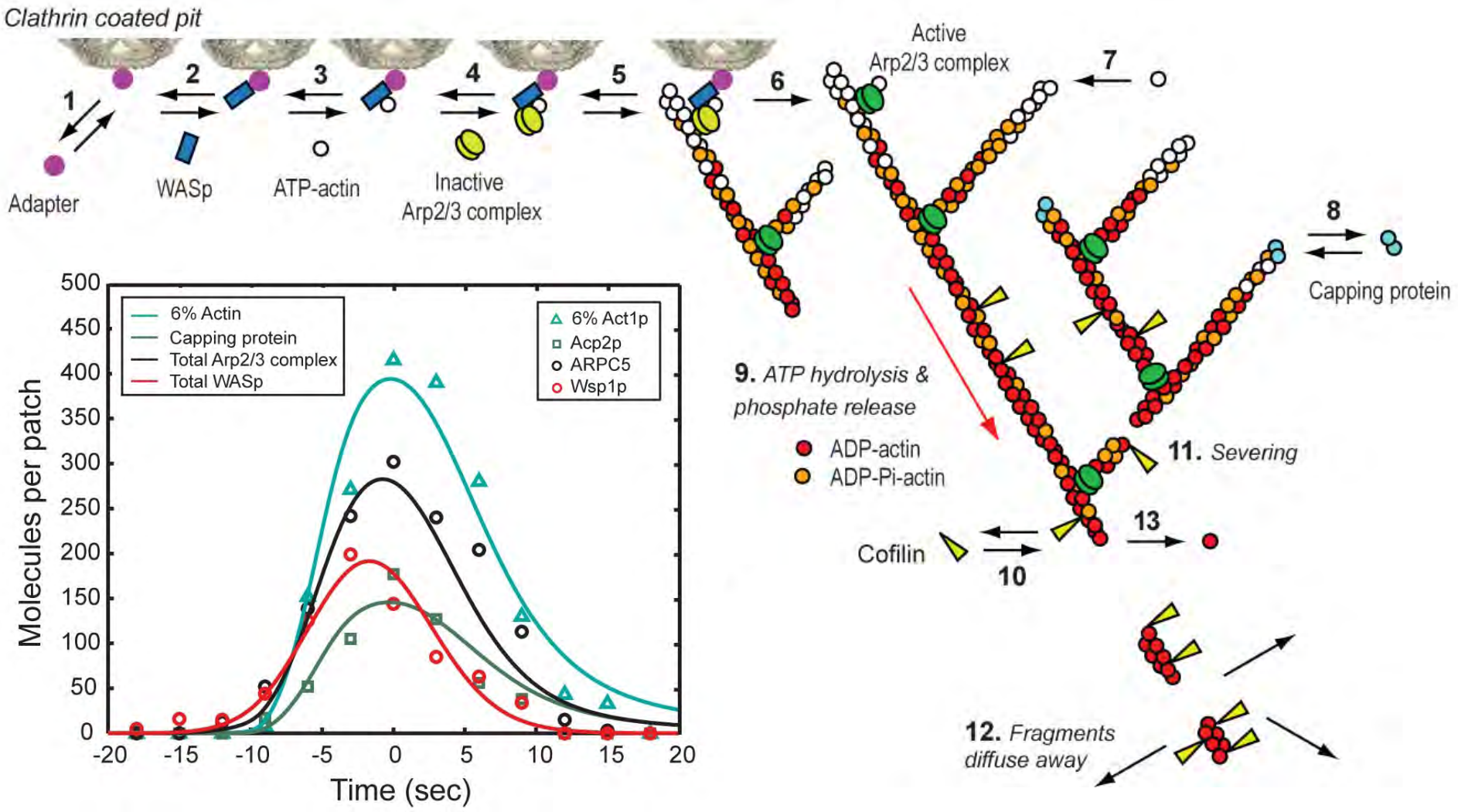


Mechanism is robust: assembles & disassembles actin with a wide range of parameter values

But amplitudes & times differ from cellular observations using parameters from biochemical experiments

Berro & Sirotkin, MBoC 2010

Hypothesis for assembly & turnover of actin patches



A limited set of parameter values gives simulation outputs matching cellular amplitudes & times

Berro & Sirotkin, MBoC 2010

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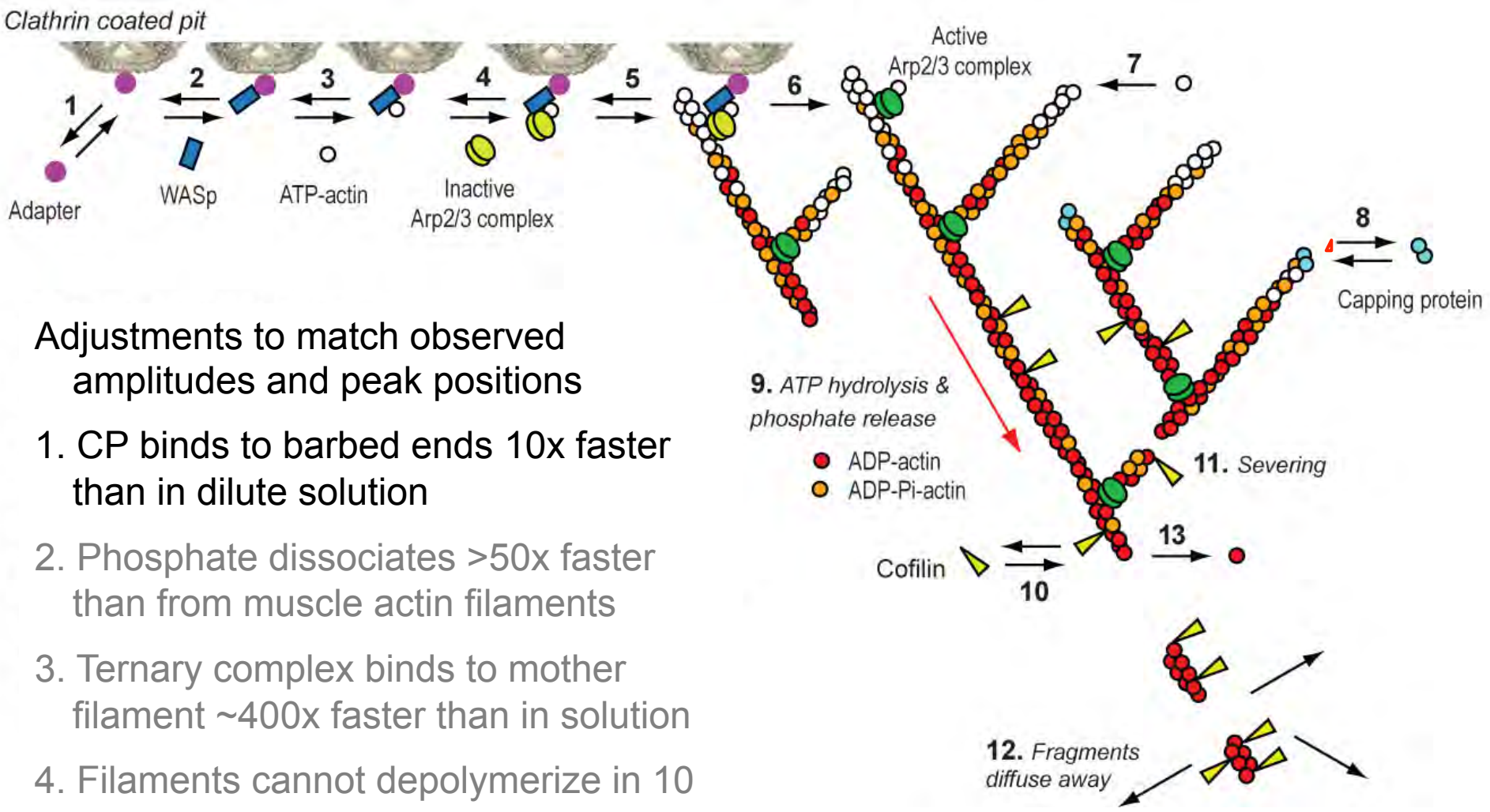

Adjustments to match observed amplitudes and peak positions

1. CP binds to barbed ends 10x faster than in dilute solution
2. Phosphate dissociates >50x faster than from muscle actin filaments
3. Ternary complex binds to mother filament ~400x faster than in solution
4. Filaments cannot depolymerize in 10 seconds, so pieces must be severed and then diffuse away

Berro & Sirotkin, MBoC 2010

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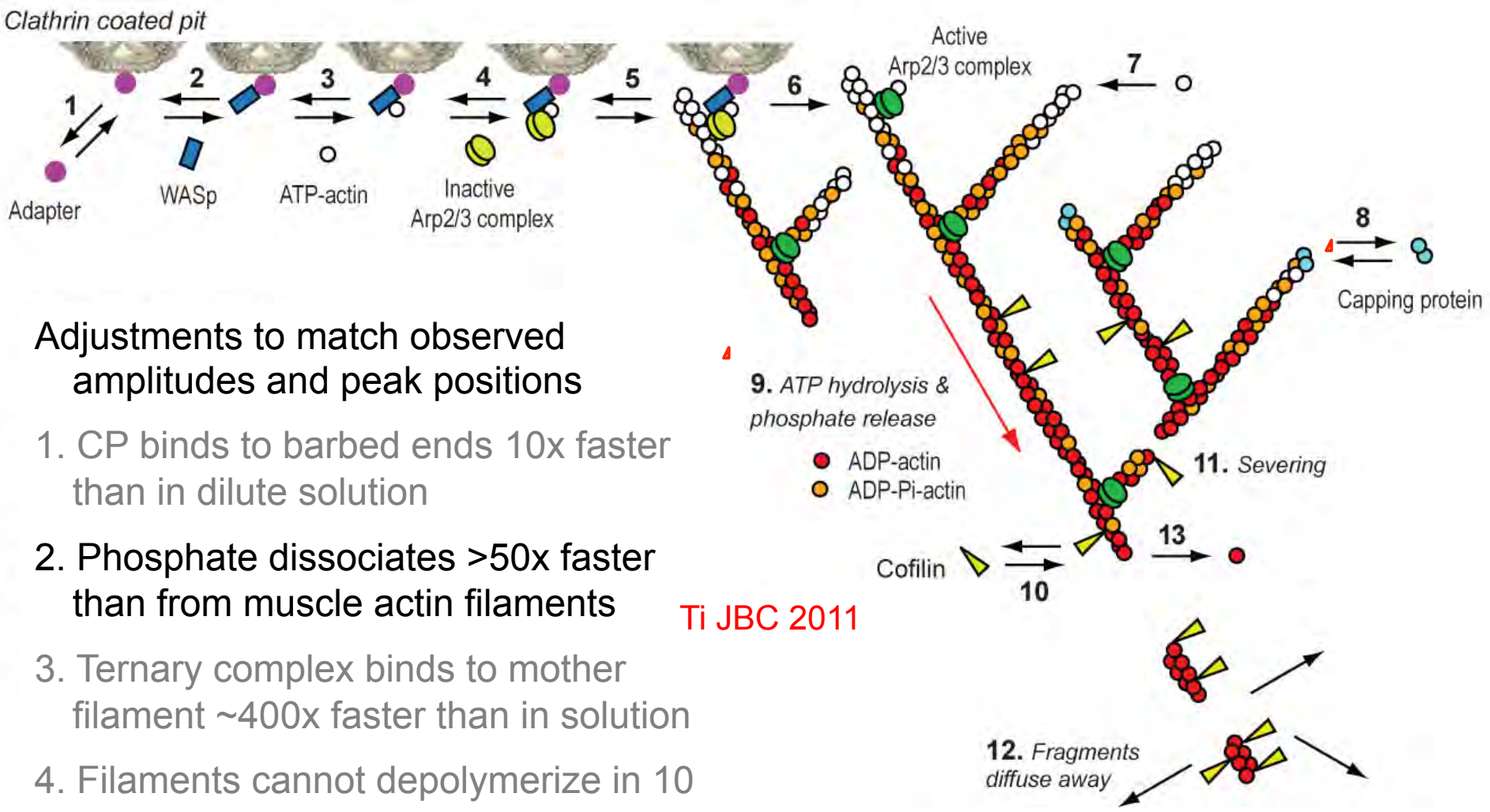

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Ti JBC 2011

Berro & Sirotkin, MBoC 2010

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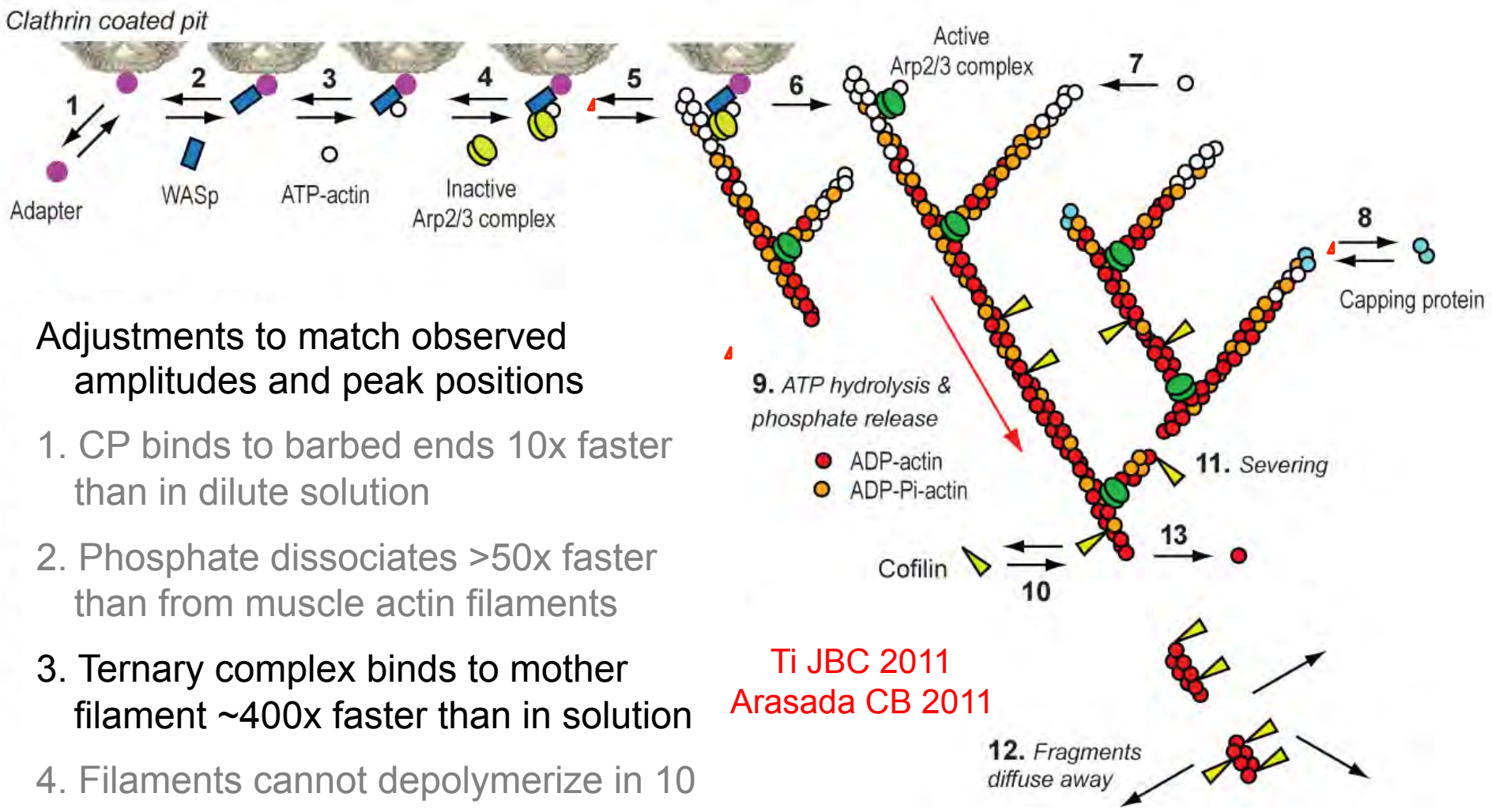

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Ti JBC 2011
Arasada CB 2011

Berro & Sirotkin, MBoC 2010

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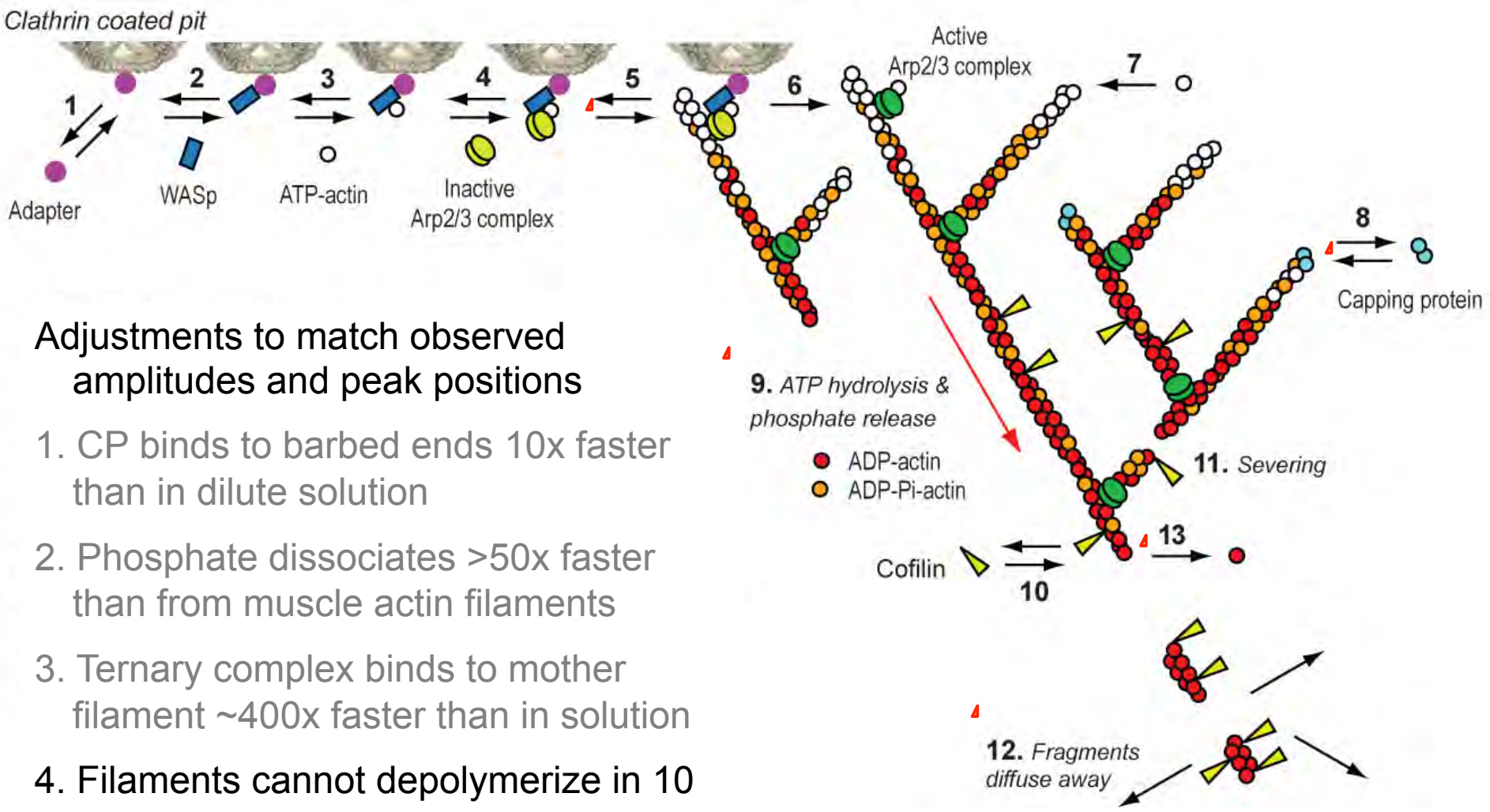

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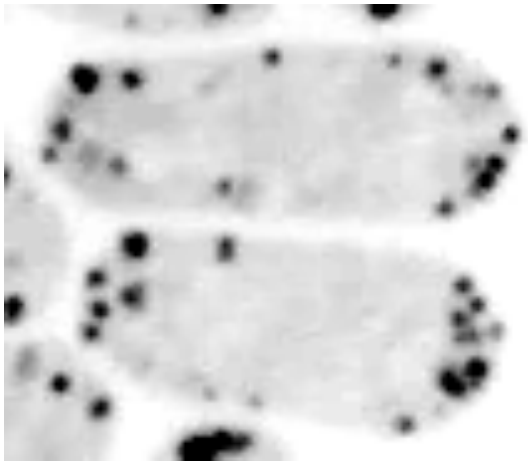
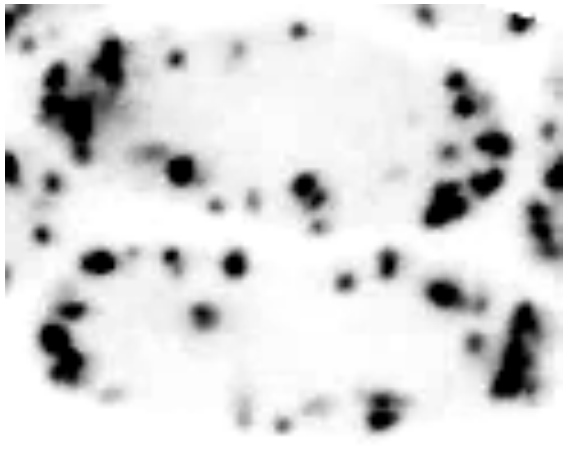
Actin patches in cells with slowly severing cofilin mutants

Actin patches tagged with fimbrin-GFP

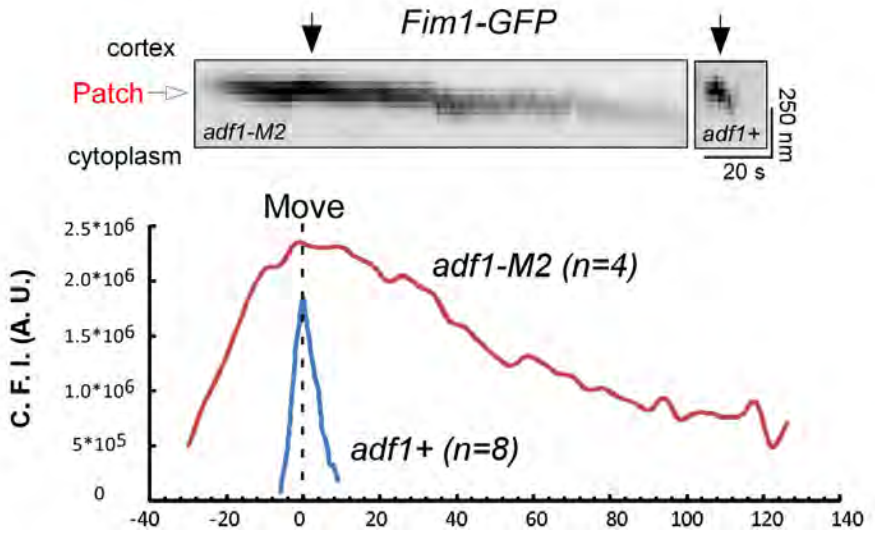
Wild type cells



Cofilin-M2 cells

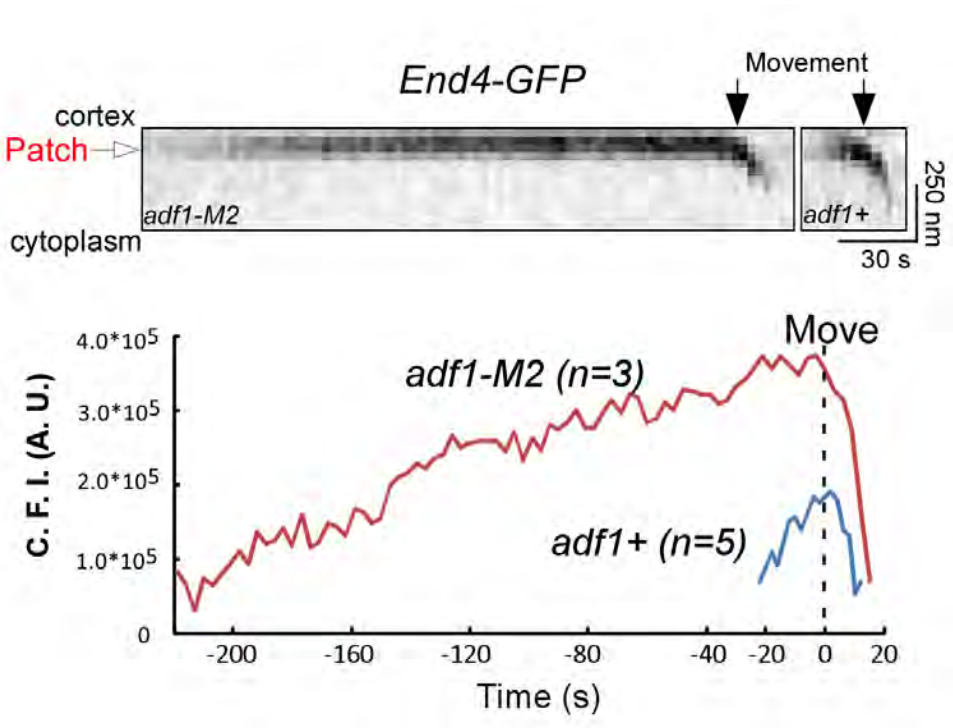


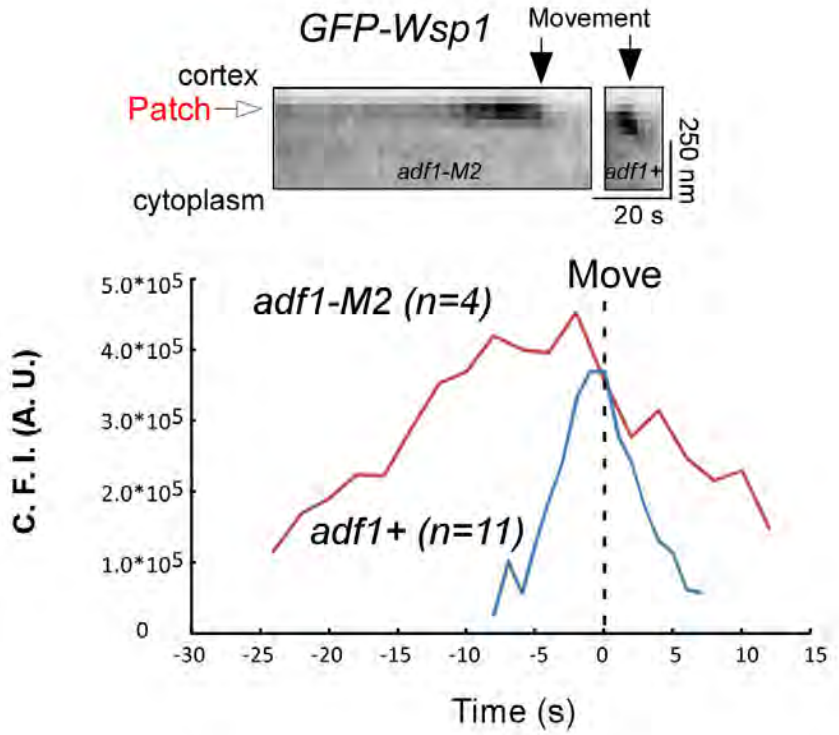
Actin patches in cells depending on cofilin mutants disassemble slowly, as expected from the role of cofilin in actin filament turnover, but they also assemble actin filaments slowly



Qian Chen

Actin patches in cells with slowly severing cofilin mutants





Actin patches in cells depending on cofilin mutants assemble components leading to Arp2/3 complex slowly. Thus the system must include unanticipated feedback loops.

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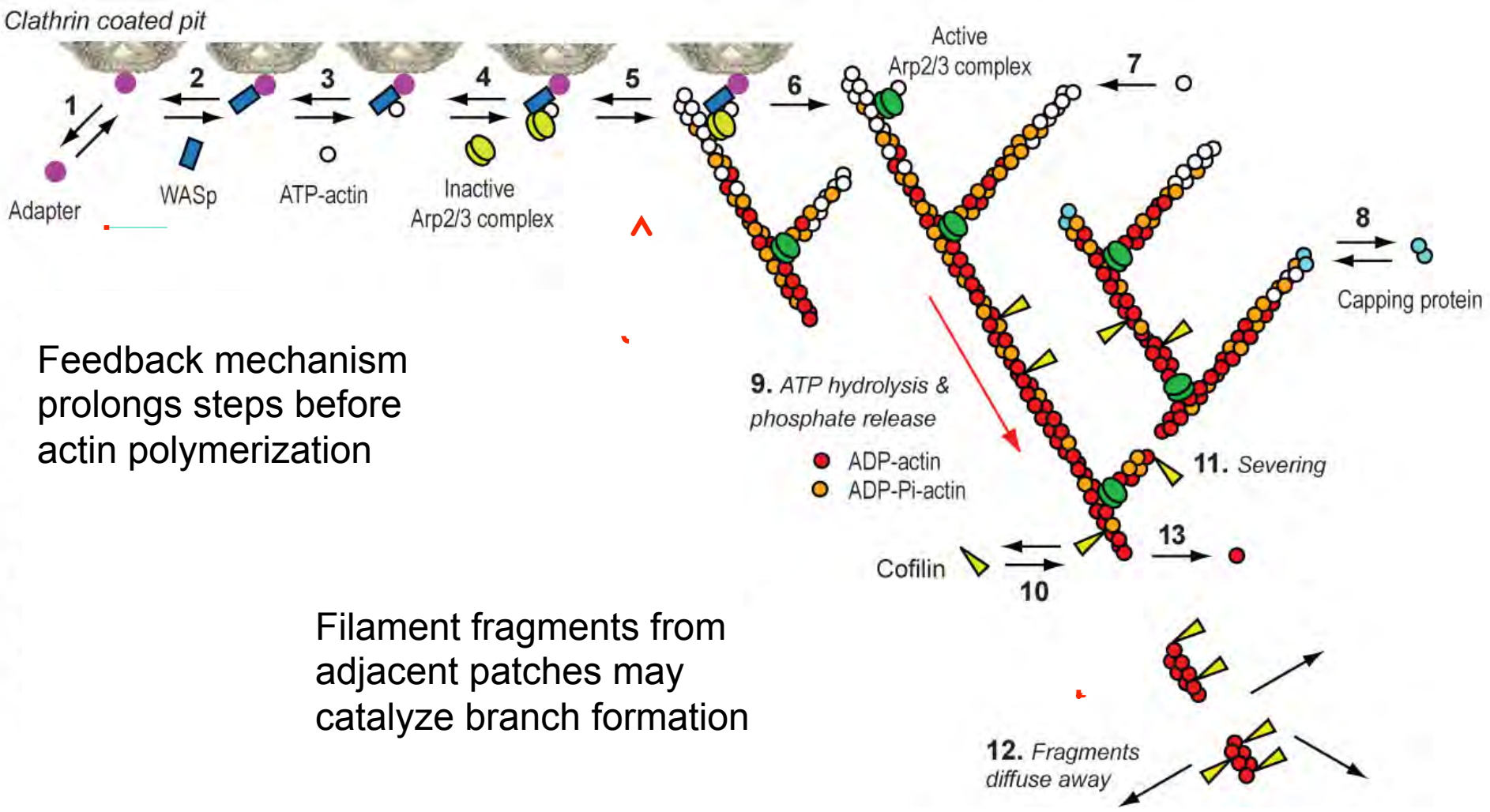
Hypothesis for assembly & turnover of actin patches



Feedback mechanism prolongs steps before actin polymerization

Filament fragments from adjacent patches may catalyze branch formation

Berro & Sirotkin, MBoC 2010