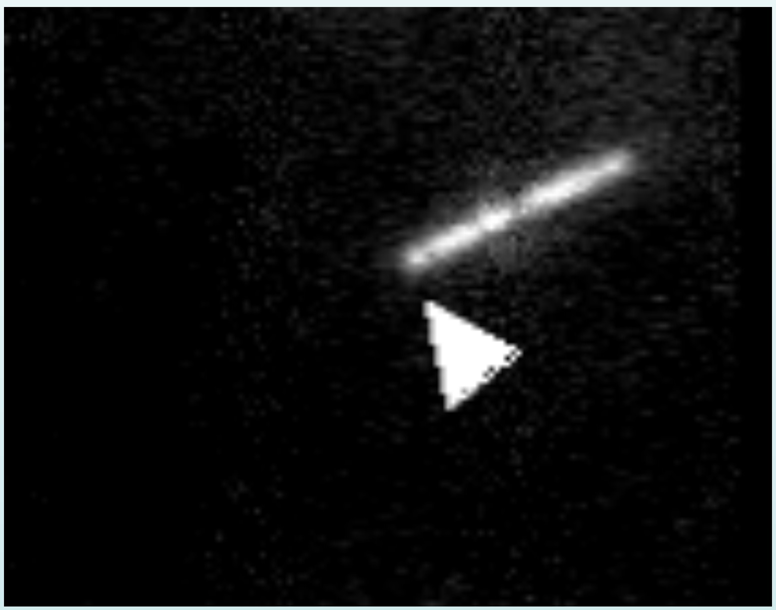
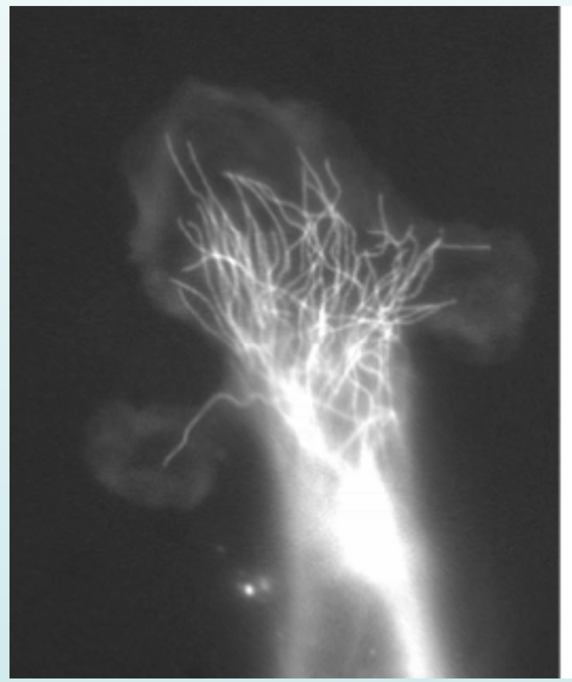
Controlling Microtubules Through Severing

Jennifer Ross

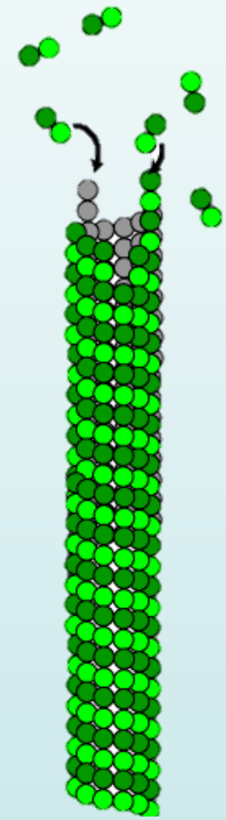
Department of Physics, University of Massachusetts Amherst



Dynamic Instability of Microtubules in vitro, Ross Lab



Dynamic Instability of Microtubules in mammalian cells, Wadsworth Lab



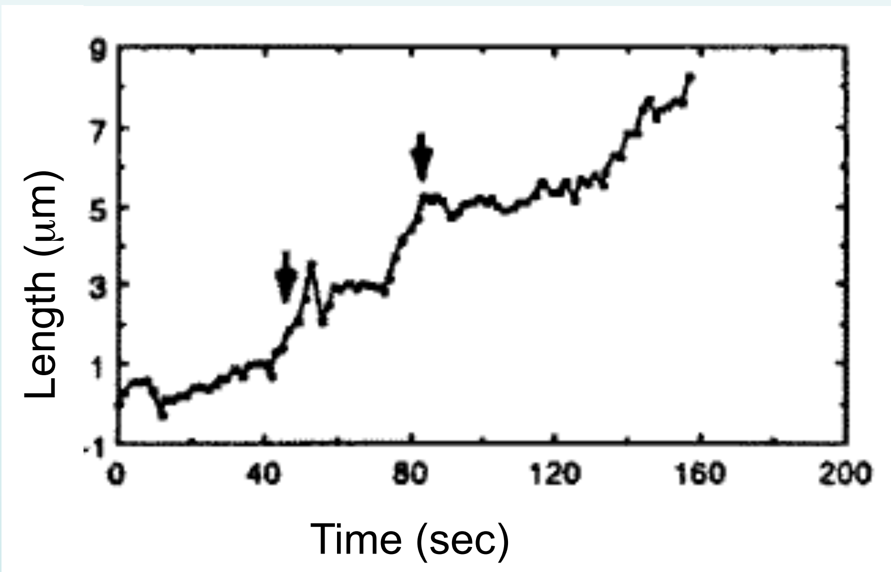
8:30am, TUESDAY, August 2, 2011

Dynamics In and Out of Cells…

Dynamic Instability occurs in vitro and in vivo

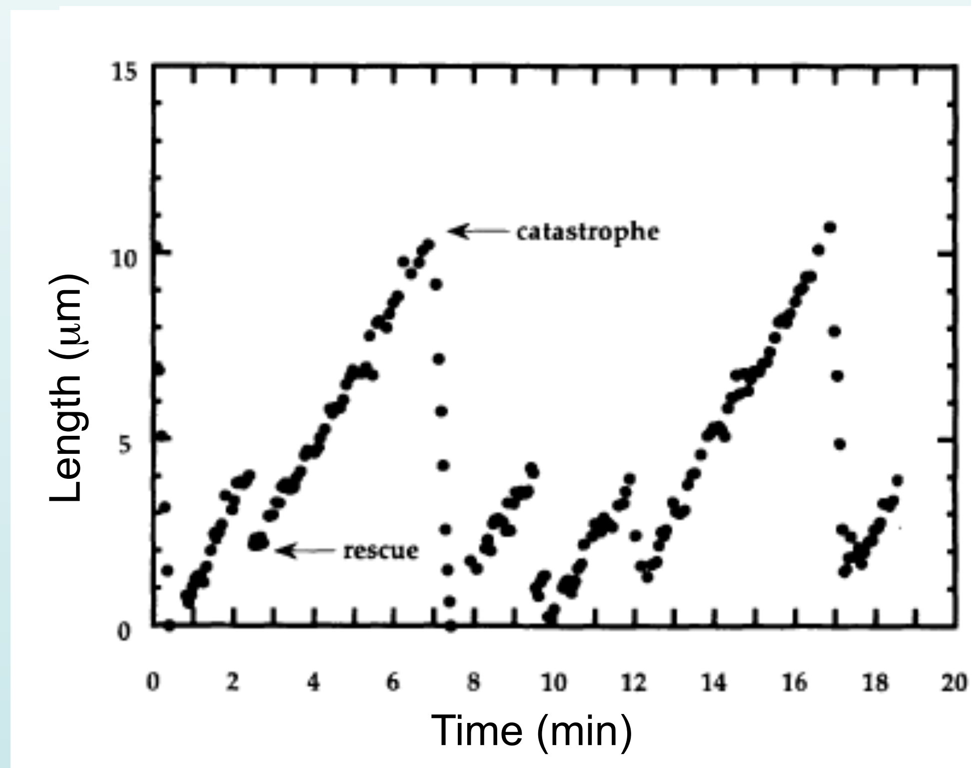
And yet… the actual "dynamics" are not the same

In vivo



Sheldon, Wadsworth, JCB, (120) 1993.

In vitro



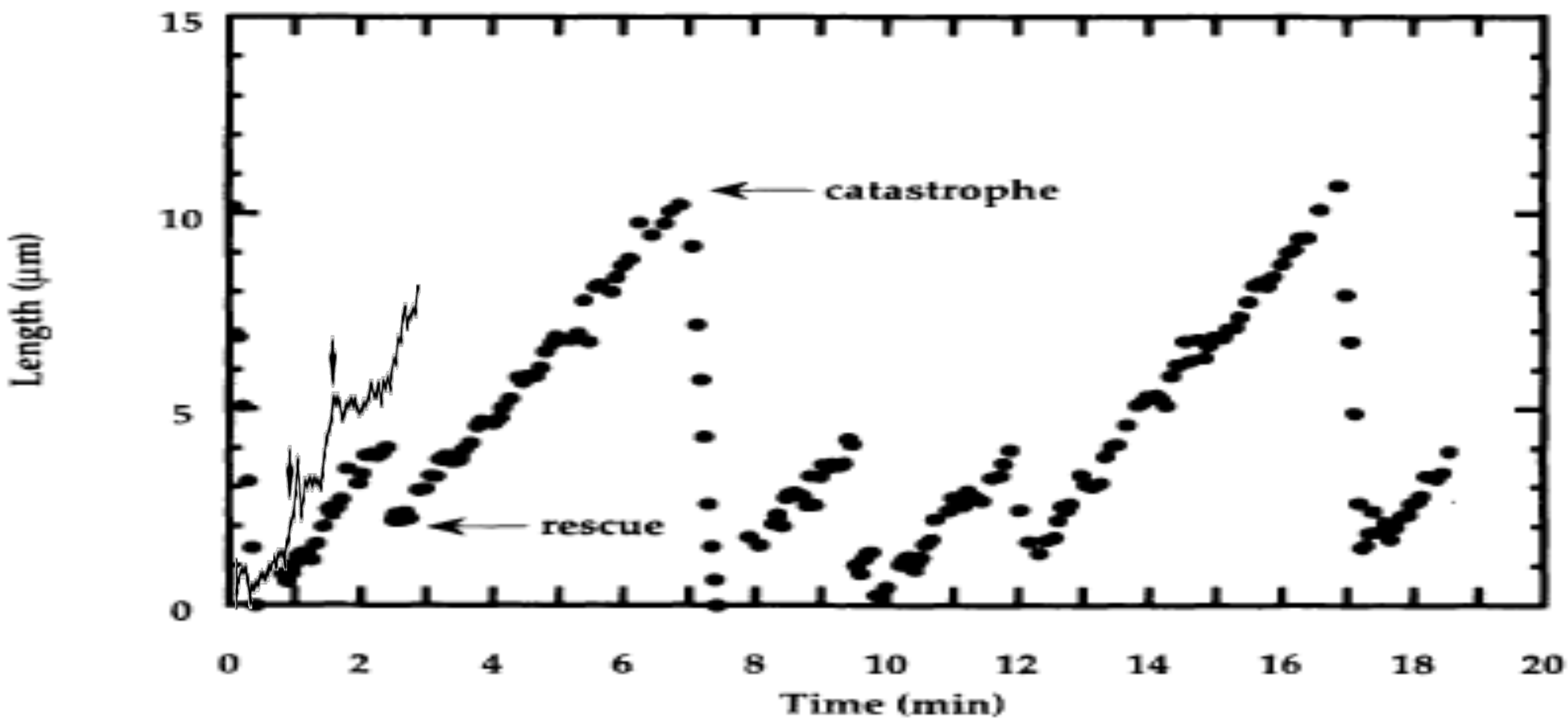
Fygenson, Braun, Libchaber, PRE, (50) 1994.

Cellular Factors Must Regulate Dynamics

In vivo, microtubules are more controlled

Microtubule Associated Proteins (MAPs) regulate:

Nucleation, polymerization, depolymerization, stabilization, destabilization



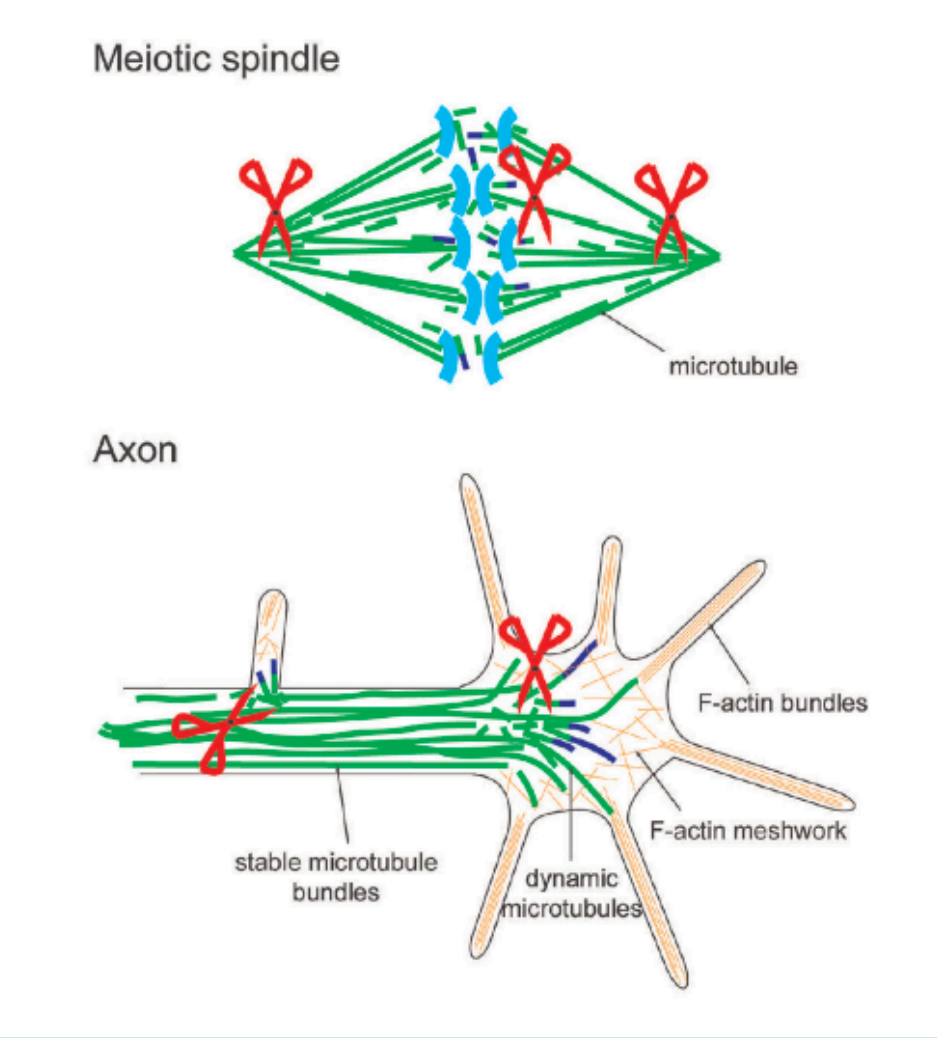
Why is Severing Important?

Severing enzymes can regulate:

Microtubule dynamics (mitosis, meiosis, cilia)

Microtubule organization (interphase, mitosis, axons, cilia)

Microtubule density (meiotic spindle, axons)



Roll-Mecak and Vale, JCB Comment, 2006

Microtubule Severing Enzymes

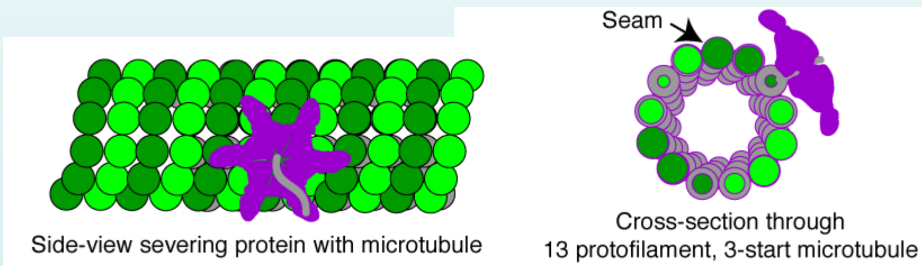
Regulate by cutting microtubules along their length

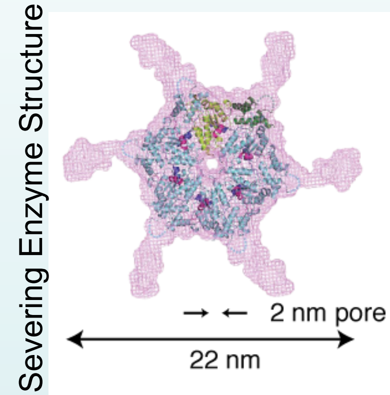
AAA+ (ATPases Associated with various cellular Activities)

We will discuss two:

Katanin

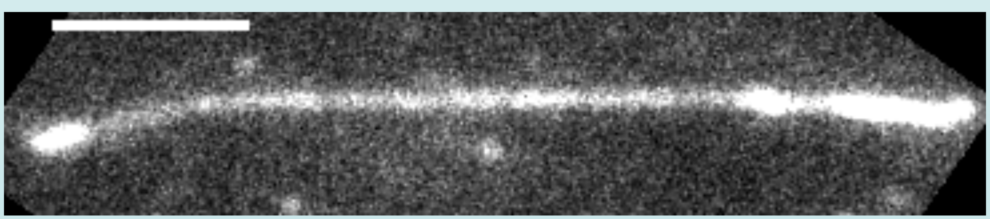
Fidgetin (newest member of the family)





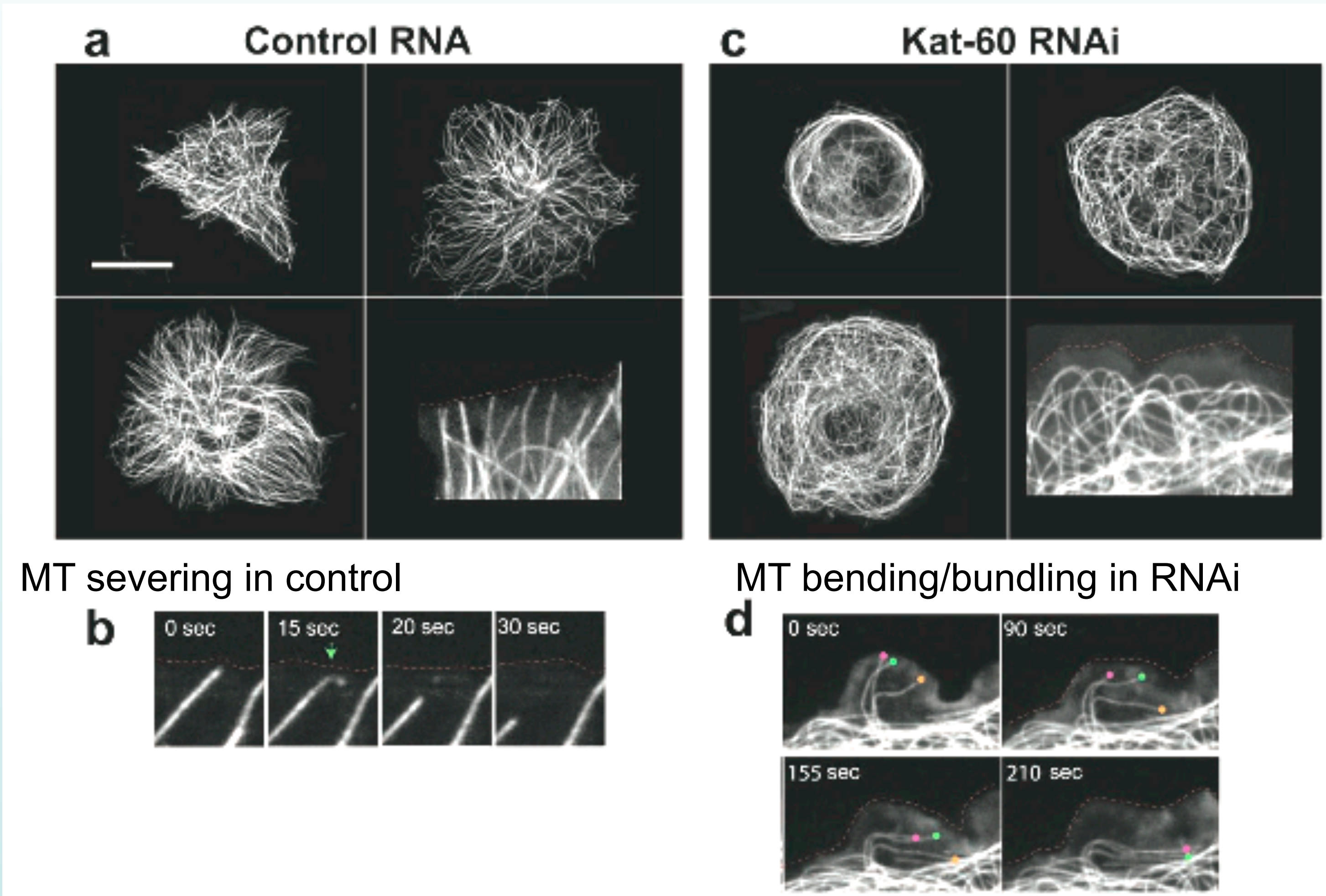
Roll-Mecak, Vale, Nature, (451) 2008

Katanin - first member identified



Katanin Controls Microtubule Network in Cells

Katanin RNAi alters microtubule length and localization

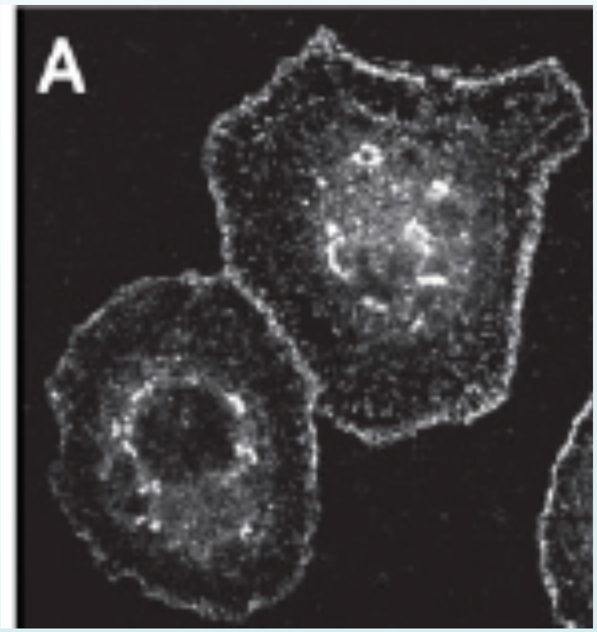


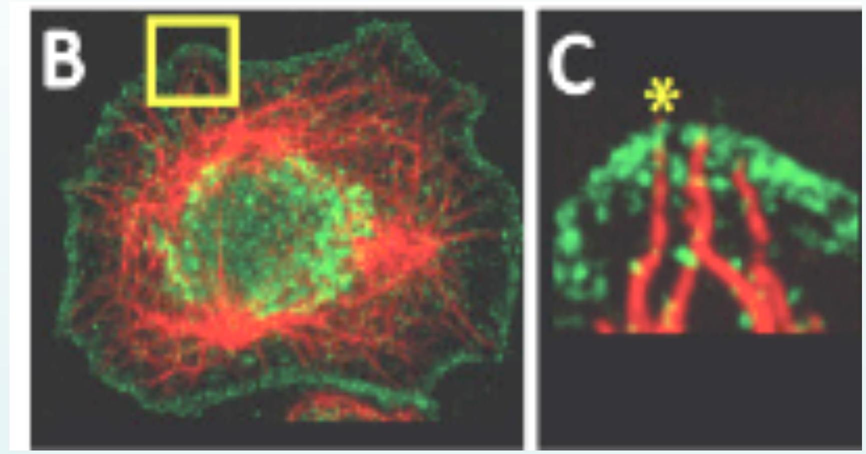
Zhang, Grode, Stewman, Diaz, Liebling, Curie, Buster, Asenjo, Sosa, **JLR**, Ma, Rogers, Sharp, Nature Cell Biology, 2011.

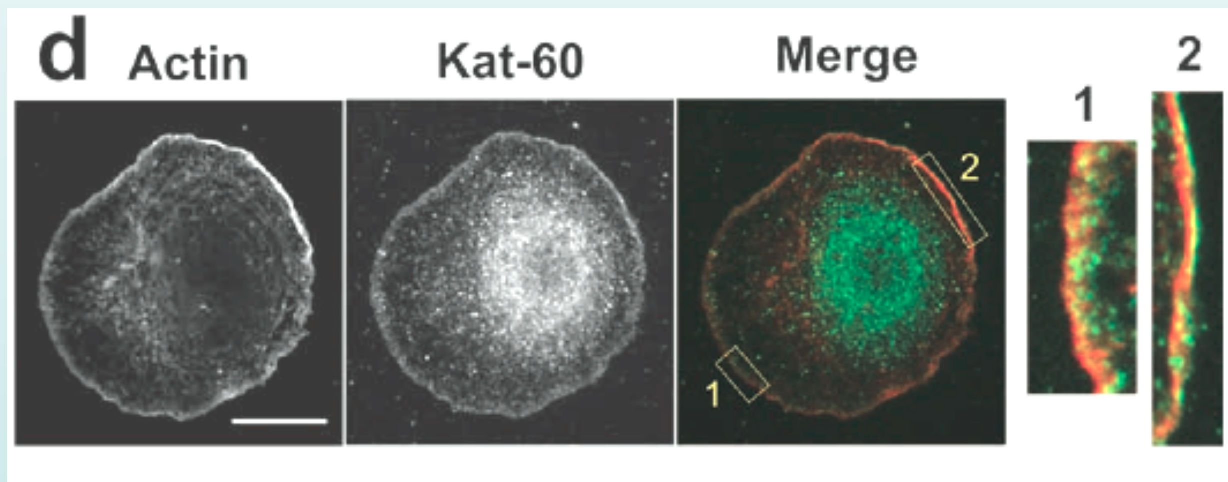
Katanin Localizes to the Cortex

Katanin at the cortex of S2 cells

Localizes with Actin cortex



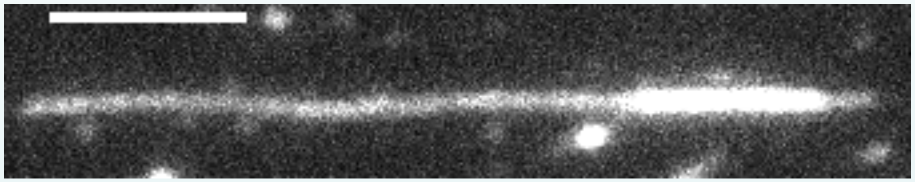




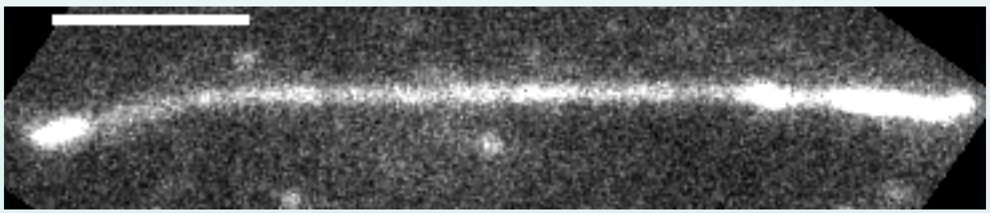
Zhang, Grode, Stewman, Diaz, Liebling, Curie, Buster, Asenjo, Sosa, **JLR**, Ma, Rogers, Sharp, Nature Cell Biology, 2011.

Katanin Severing Activity

without Katanin Control

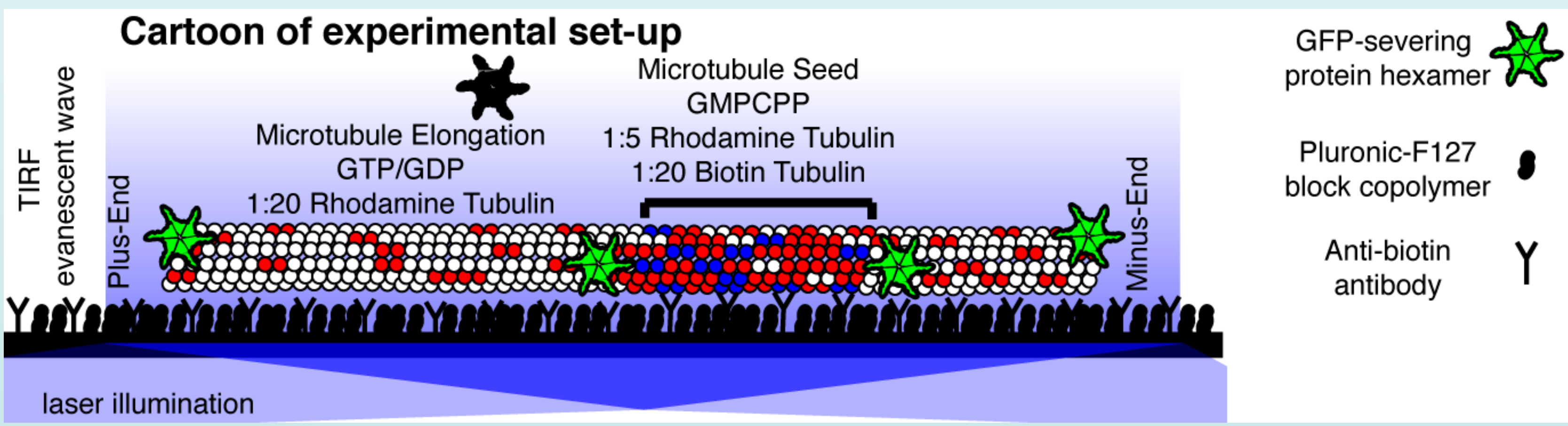


with Katanin



Filaments stabilized with taxol

Count severing events per length per second



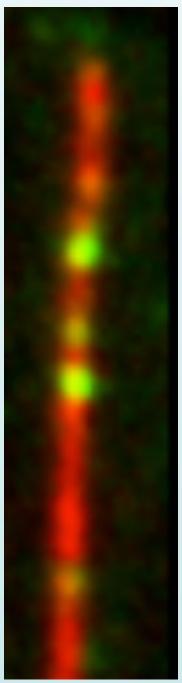
Diaz, Morelli, Bailey, Zhang, Sharp, **JLR**, Biophysical Journal (2011)

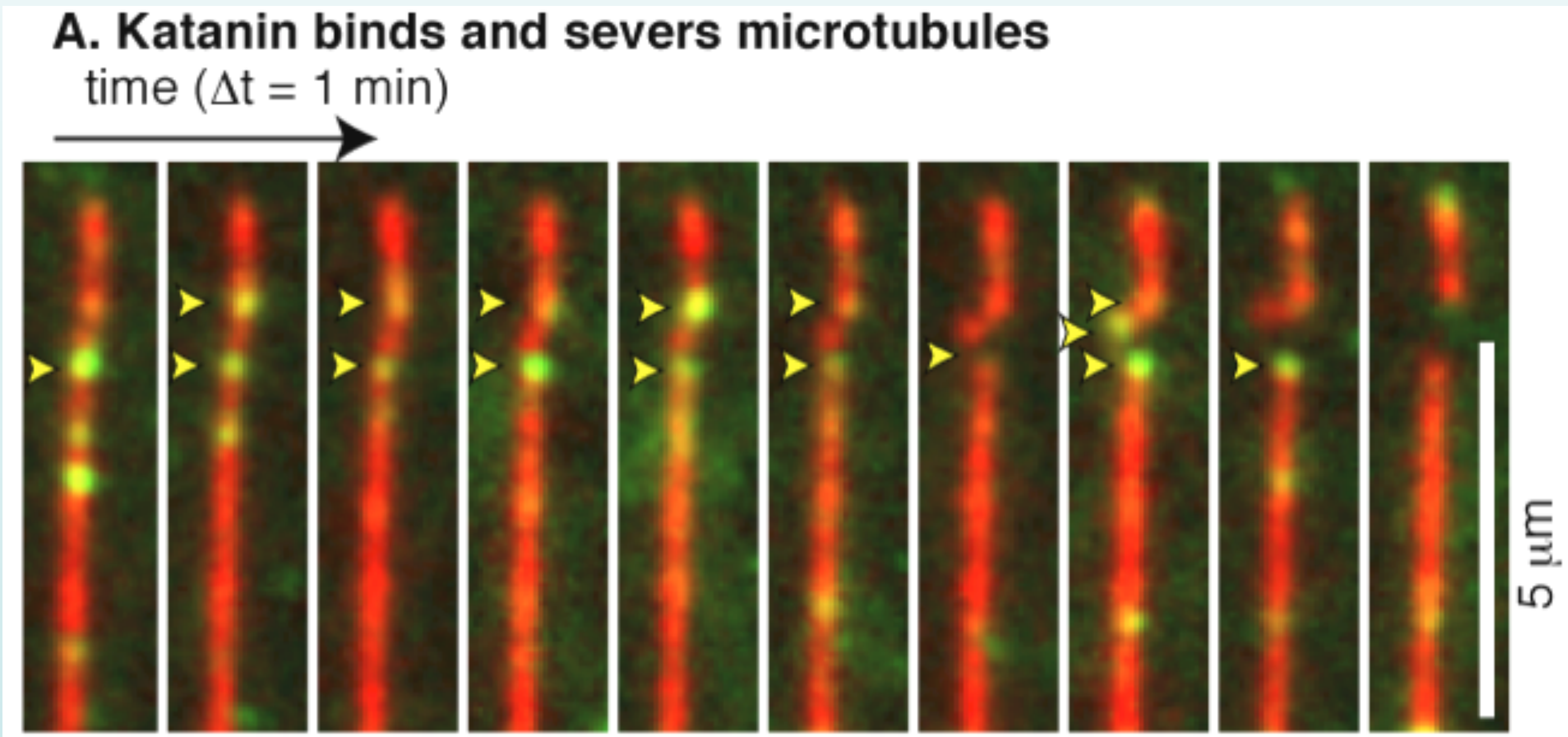
Katanin Binds to Cut

Binding to specific sites

Severing occurring at these sites

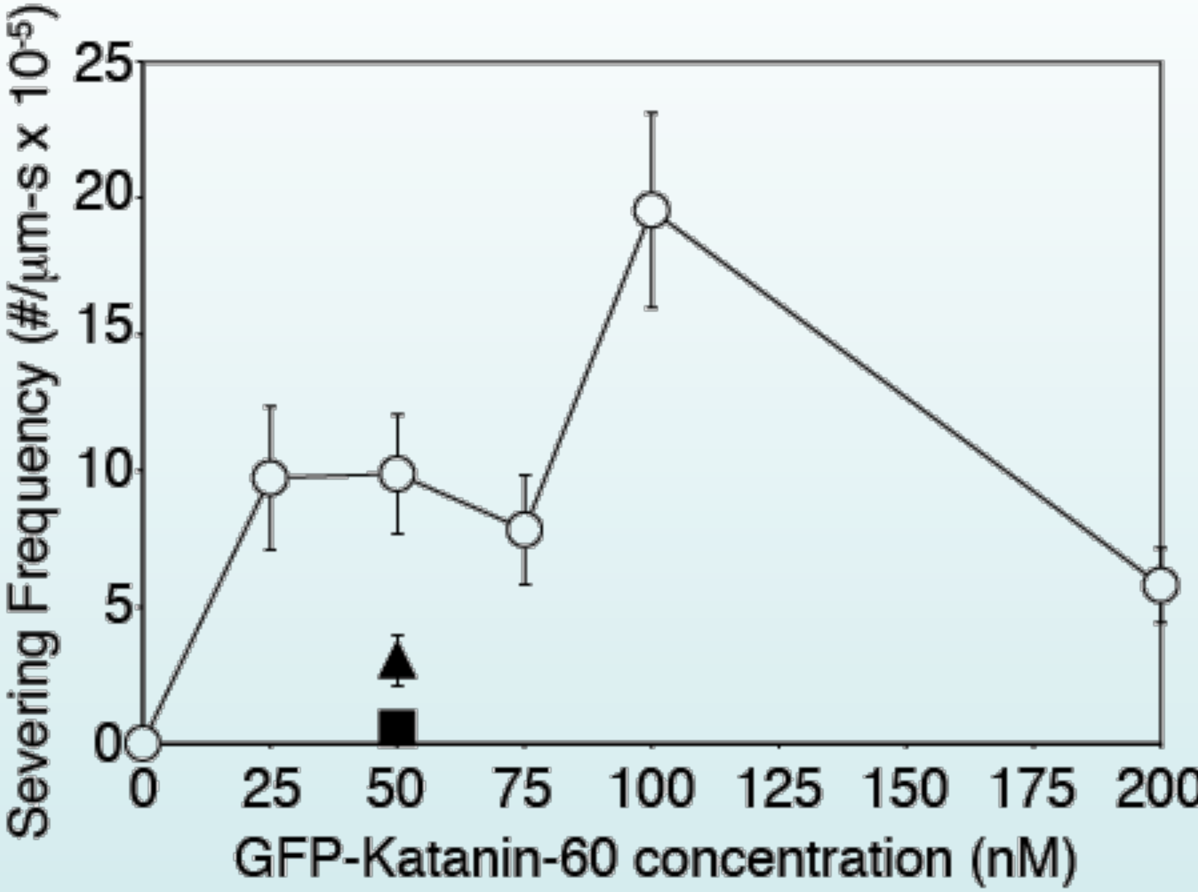
Binding to ends





Diaz, Morelli, Bailey, Zhang, Sharp, **JLR**, Biophysical Journal (2011)

Severing Depends on Katanin Concentration



<u>Severing Frequency</u> = #severing events per length of microtubule per time of observation

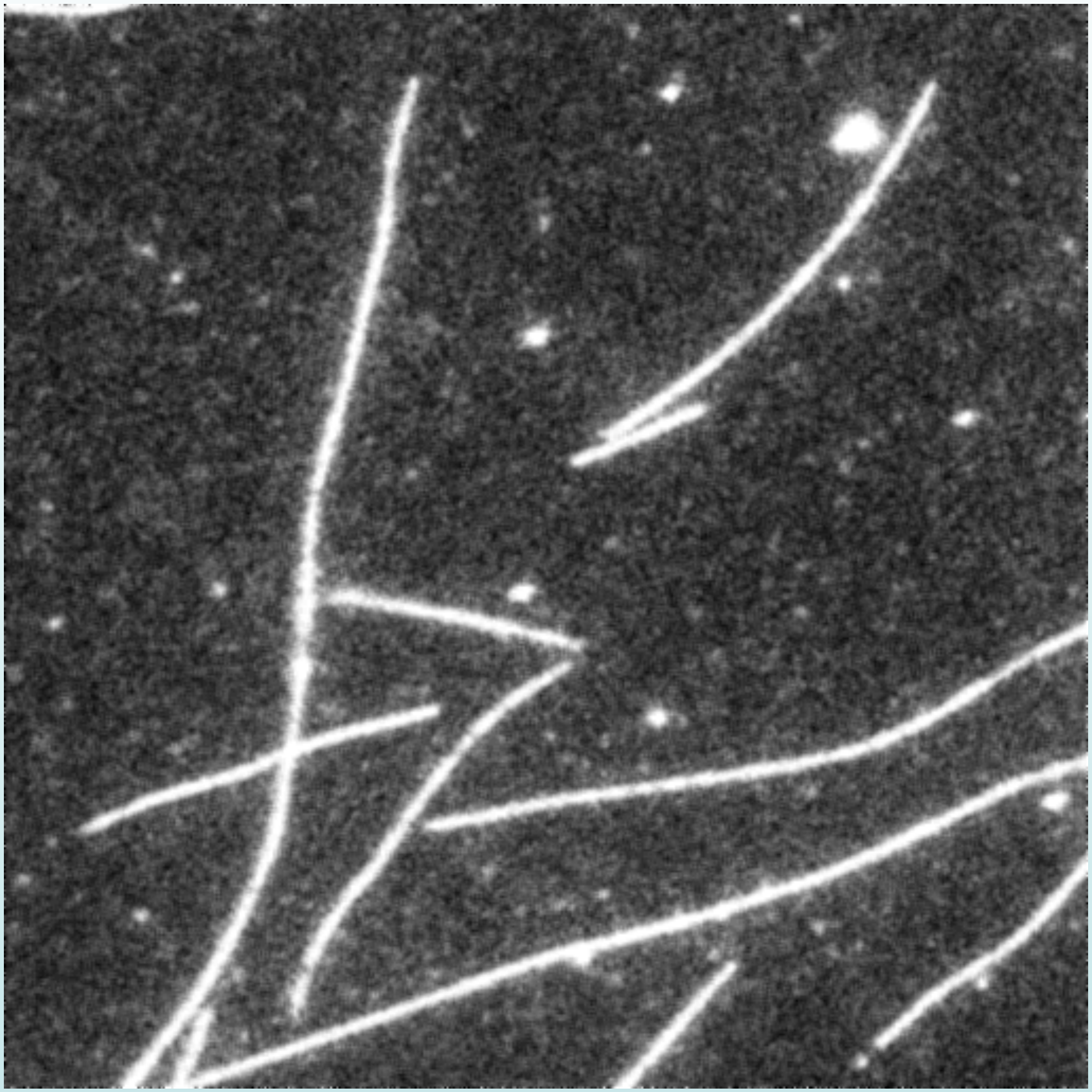
Severing frequency increases with increasing Katanin.

Protein aggregates at higher concentrations

Diaz, Morelli, Bailey, Zhang, Sharp, **JLR**, <u>Biophysical Journal</u> (2011)

New Mode of Operation: Depolymerization

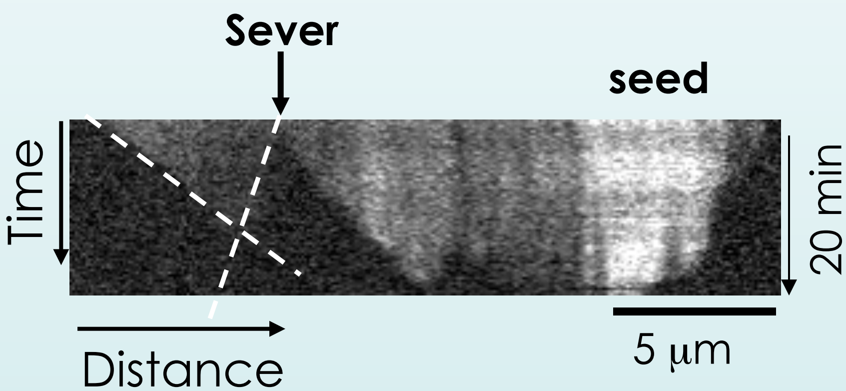
These are taxol-stabilized microtubules!



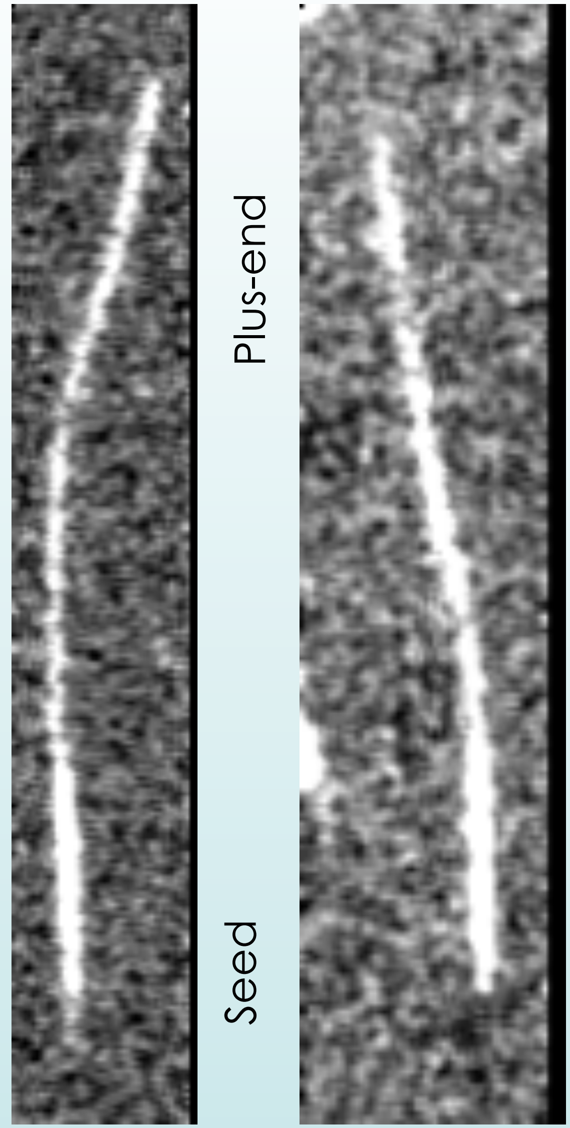
Depolymerization Rate Depends on Polarity

We measured two different depolymerization rates for each microtubule.

Using polarity-marked microtubules, fast rate at the plus-end.

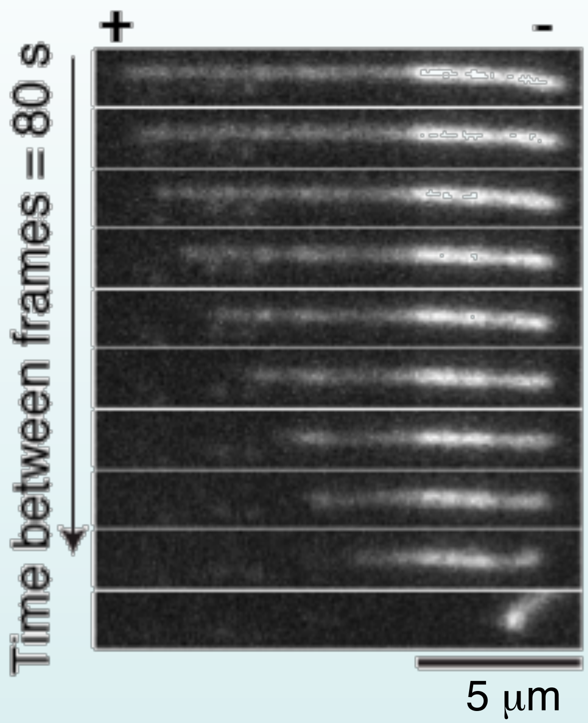


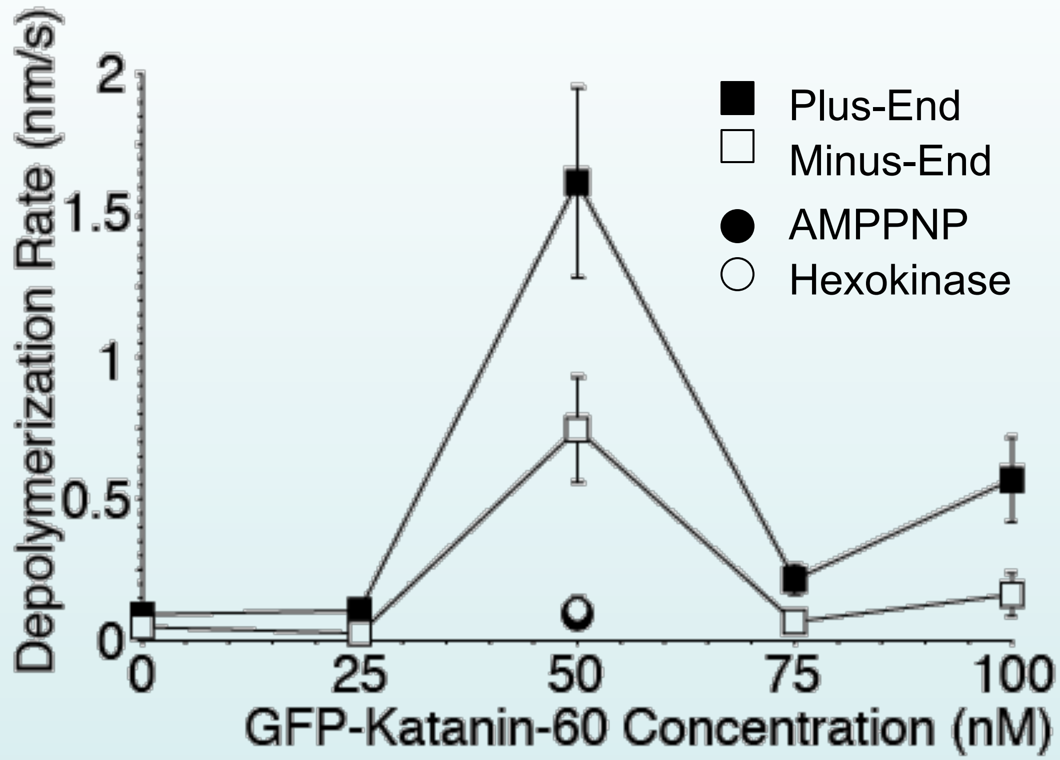
Kymograph = space-time plot



Zhang, Grode, Stewman, Diaz, Liebling, Curie, Buster, Asenjo, Sosa, **JLR**, Ma, Rogers, Sharp, Nature Cell Biology, 2011.

Depolymerization Best at Lower Concentrations





Depolymerization rate peaks at lower concentration (50 nM) than severing (100 nM)

Diaz, Morelli, Bailey, Zhang, Sharp, **JLR**, Biophysical Journal (2011)

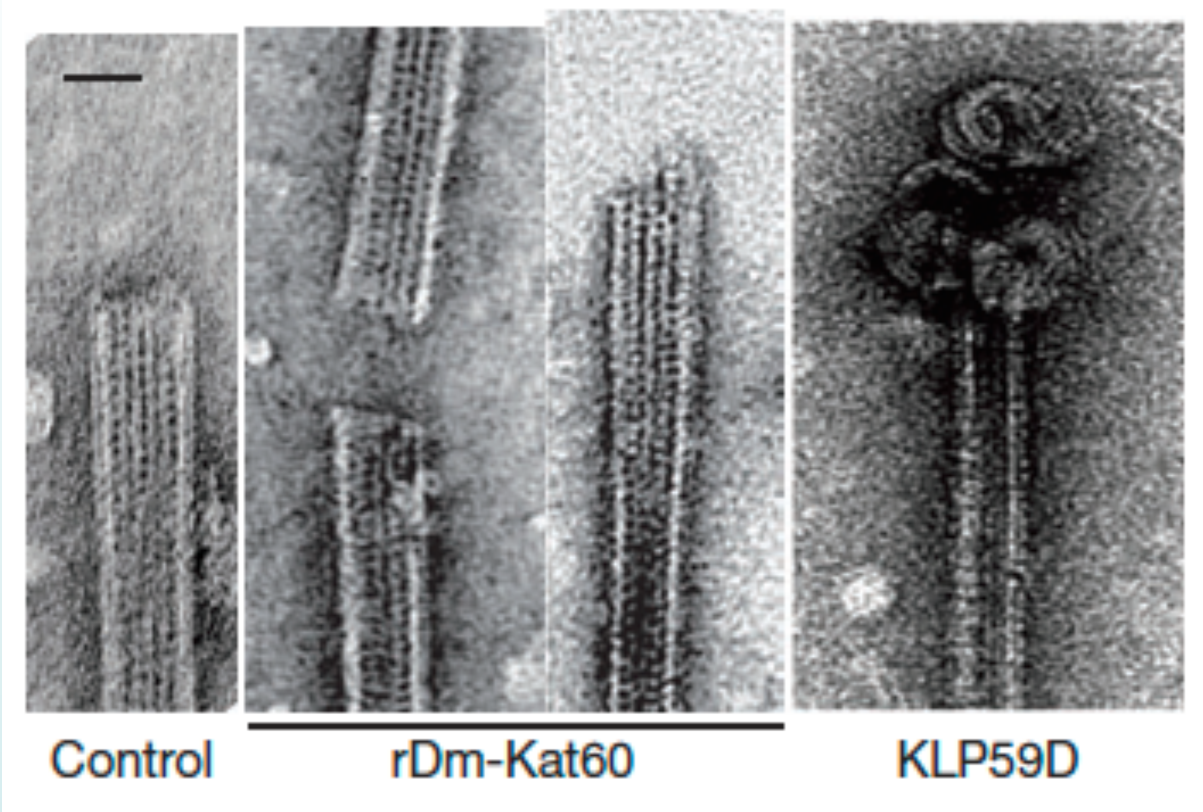
Depolymerization = End Severing?

Negative Stain EM

Blunt microtubule ends without and with Katanin

Depolymerizing kinesins curl back protofilaments

Severing proteins maintain blunt ends



Hernando Sosa Lab

Why the end?

Lattice defects?

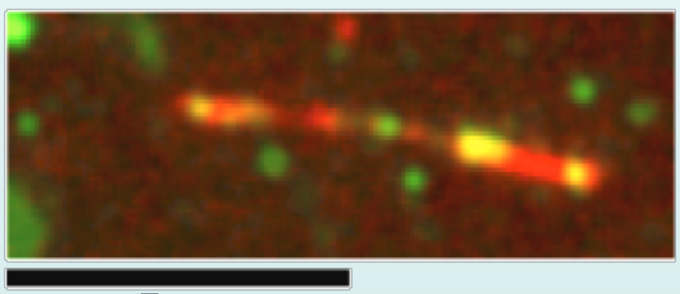
Zhang, Grode, Stewman, Diaz, Liebling, Curie, Buster, Asenjo, Sosa, **JLR**, Ma, Rogers, Sharp, Nature Cell Biology, 2011.

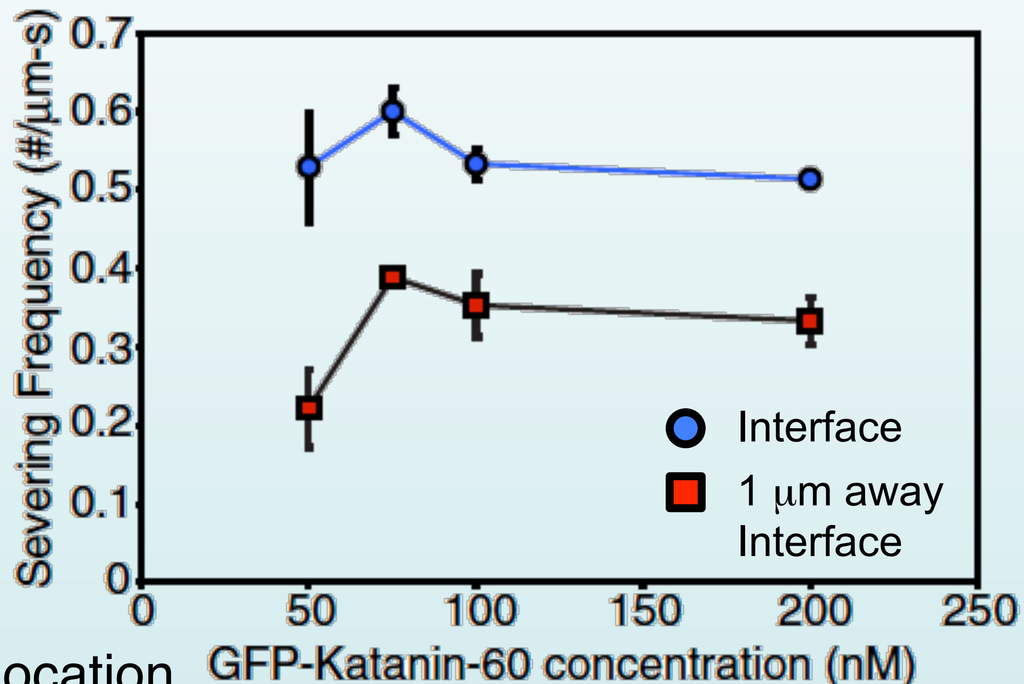
Specific Localization and Activity

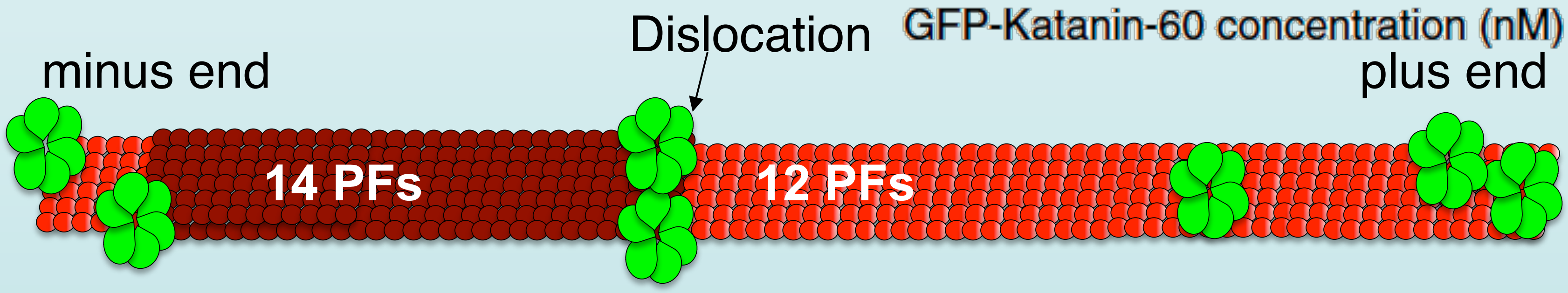
Katanin binds and severs at interface between bright, GMPCPP-seed and dim, taxol-GDP elongation segment

Prefers where tubulin conformation changes?

Defects in lattice

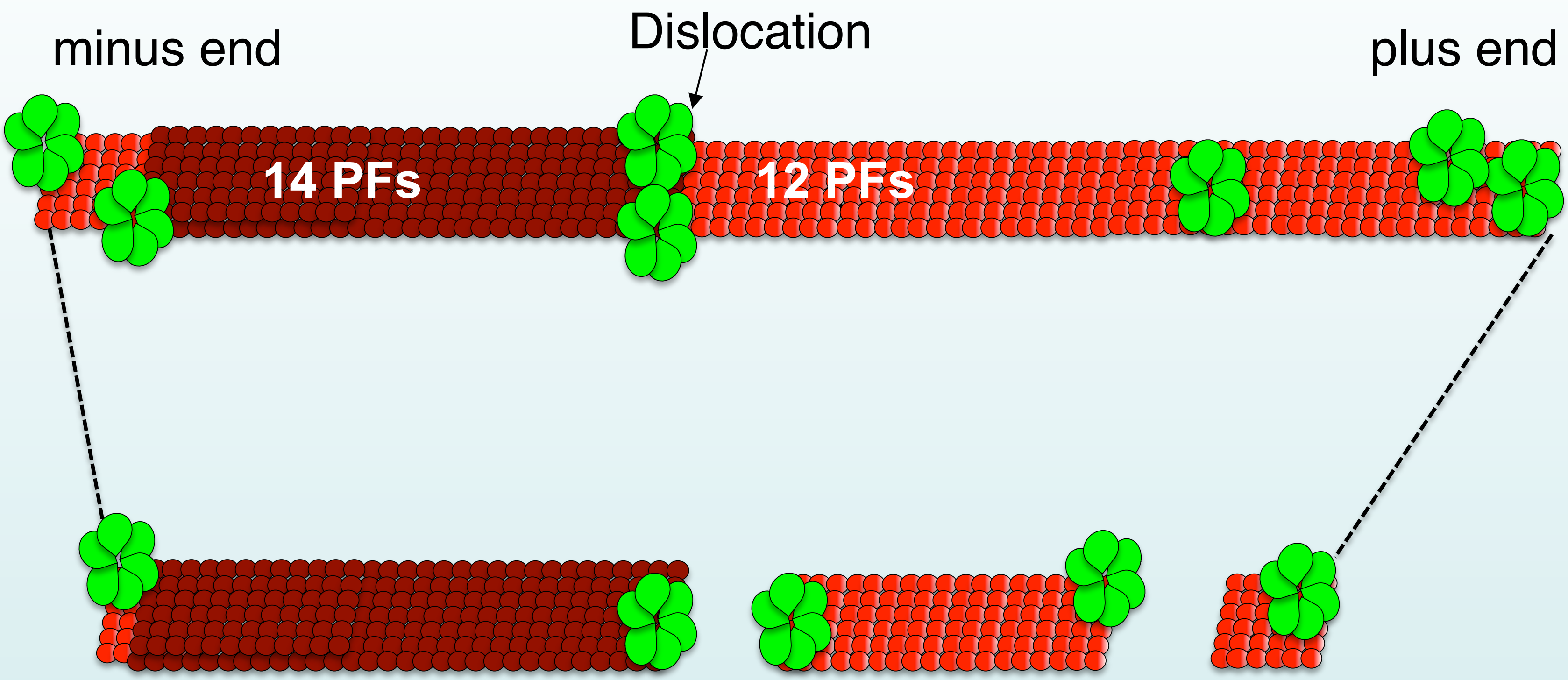






Diaz, Morelli, Bailey, Zhang, Sharp, **JLR**, Biophysical Journal (2011)

Katanin Recap



Removes tubulin from ends with preference for the plus-end

Removes tubulin from interface between GMPCPP and GDP-taxol segments

- **Either there is a dislocation or other defect at that interface**
- **Or Katanin can detect the interface between GMPCPP and GDP tubulin?**

Allegory of the Cover Art

Red dragons = microtubules with defects. Heads represent the plus-ends

Flies with swords = Drosophila katanin

Fidgetin is the Newest Severing Enzyme!

Fidget mice discovered in Jackson Labs in 1940's

In 2000, genetics showed that missing protein was AAA+ enzyme

Microtubule-philes recognized that it was similar to katanin

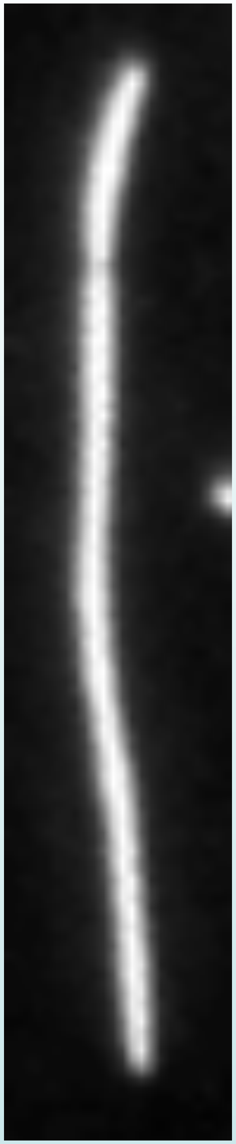
Movies courtesy of Wayne Frankel at Jackson Labs

Fidgetin knock-out mice fidget and circle

Bone and cartilage *birth defects*

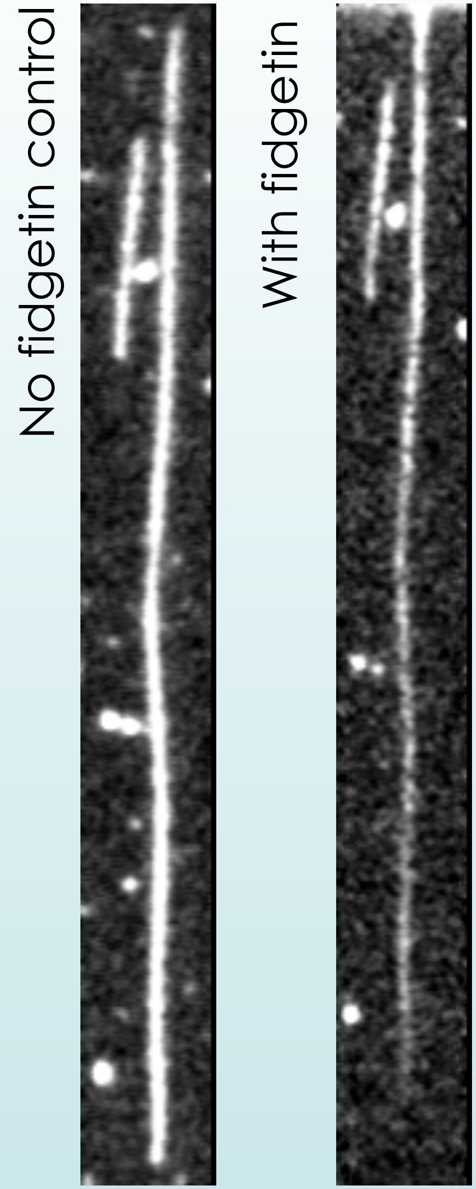
We have expressed a human construct in Sf9 cells and purified for in vitro testing…

Fidgetin Severs Microtubules!



Severs *taxol-stabilized* microtubules in vitro

First in vitro evidence that Fidgetin is a Severing Enzyme

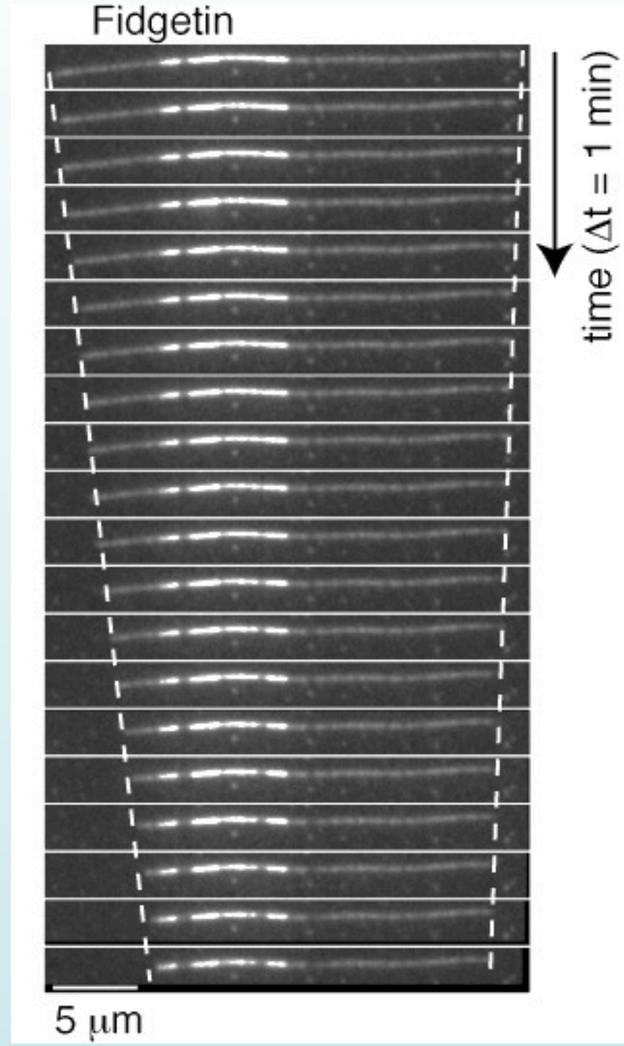


Mukherjee, et al. under review at JCB

Fidgetin Depolymerizes Microtubules

Depolymerizes *taxol-stabilized* microtubules in vitro

Which end is faster?

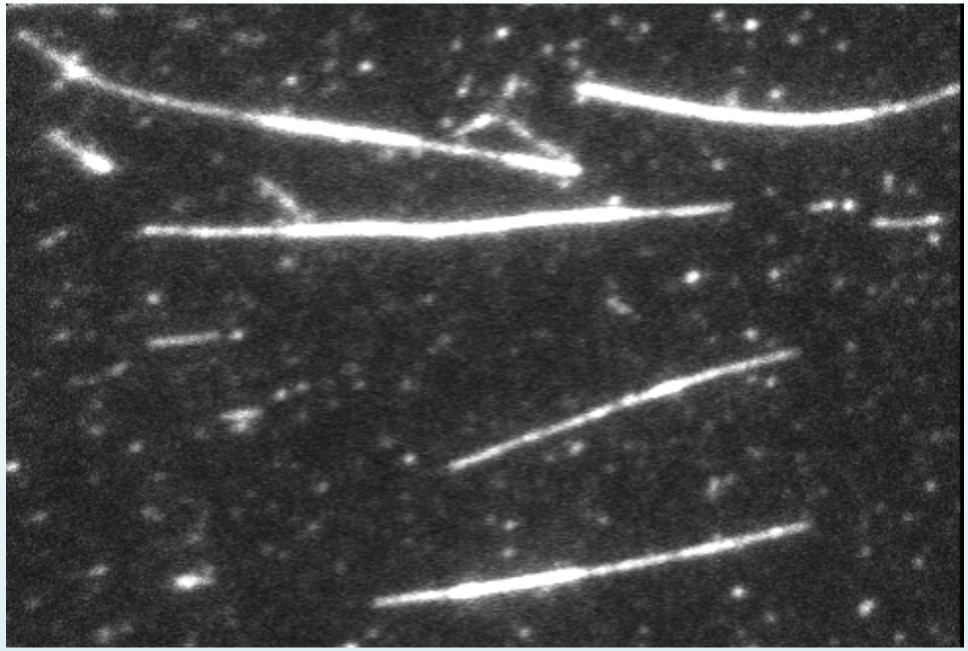


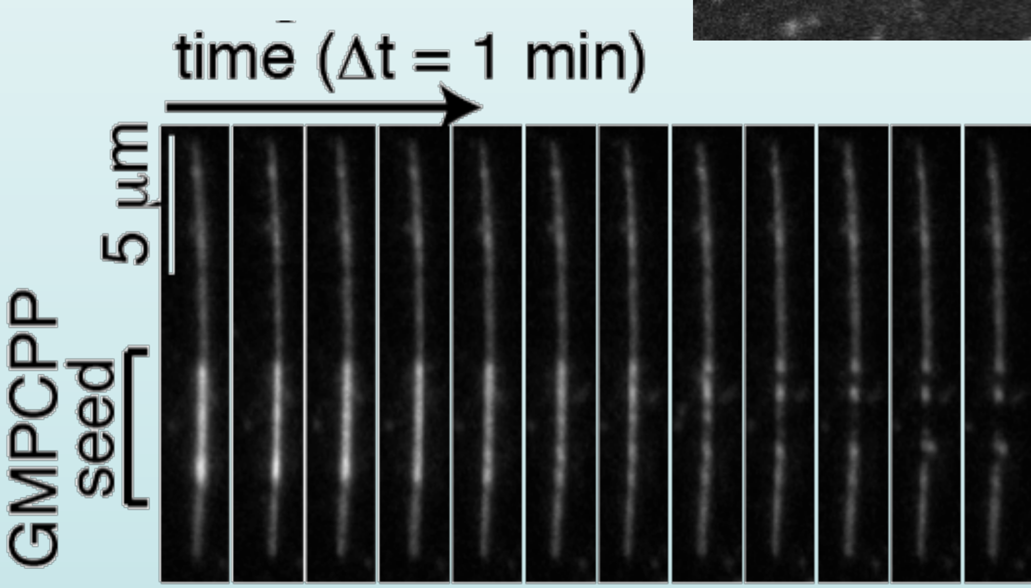
Mukherjee, et al. under review at JCB

Fidgetin Prefers GMPCPP Tubulin

Bright region = GMPCPP "seed"

Dim region = GDP-tubulin





Fidgetin removes "seed" before rest of microtubule

Can Fidgetin "read" the tubulin conformation?

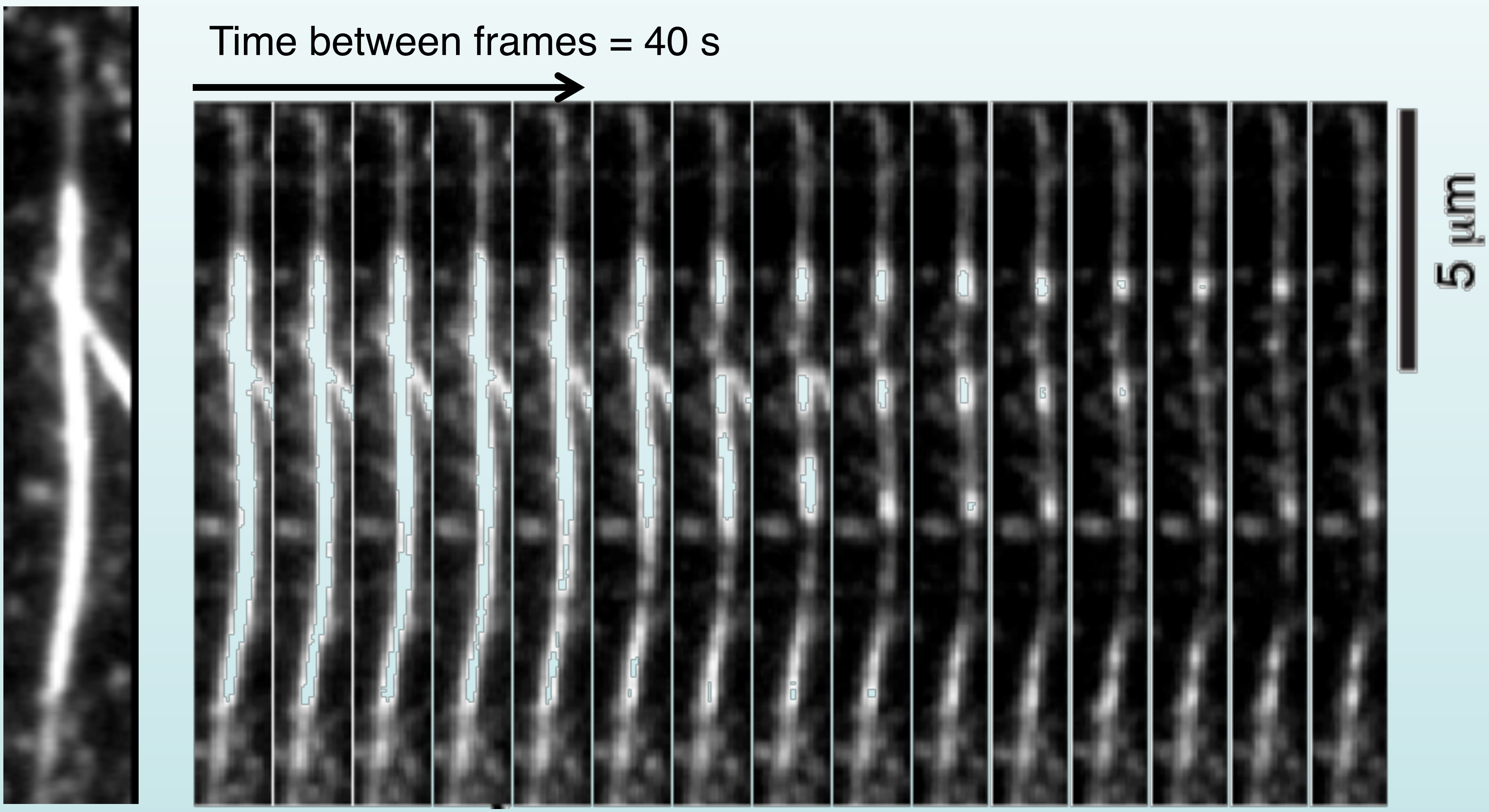
Fidgetin Strips Microtubules

Fidgetin removes protofilaments

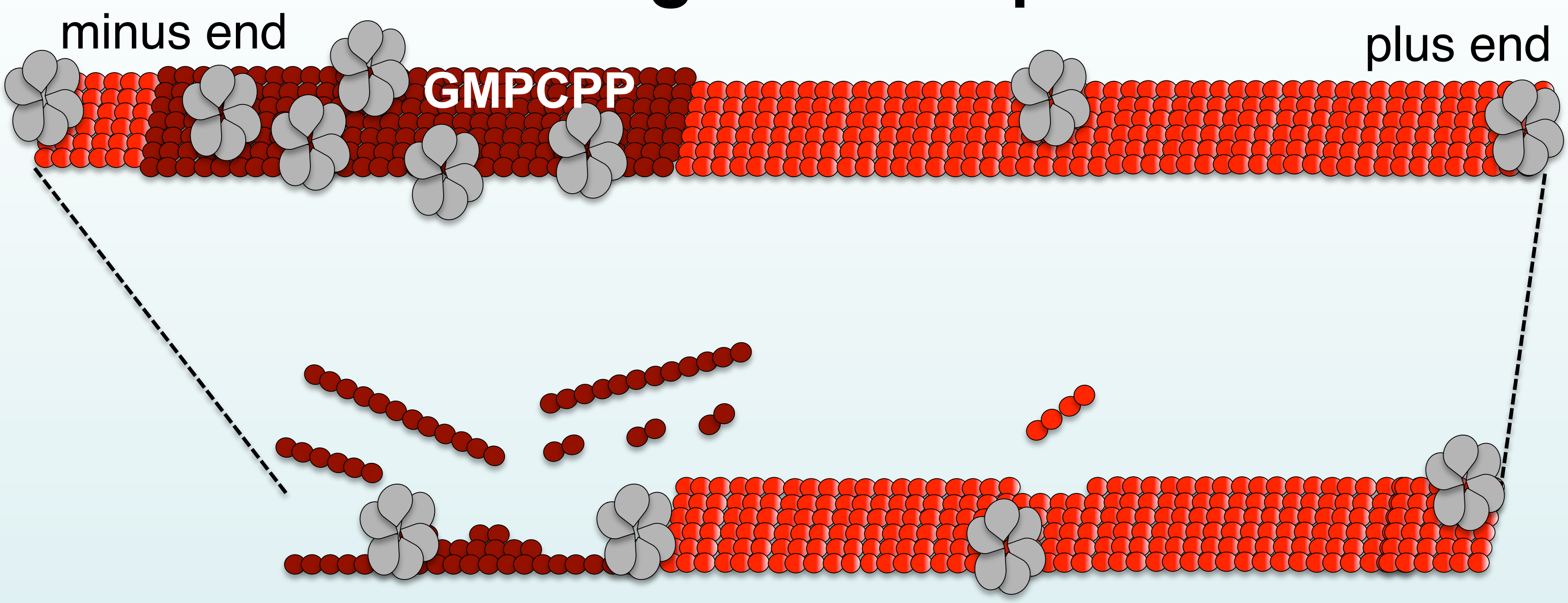
Does not cut through lattice entirely

Could be useful for lattice error correction!

Remove protofilament shifts?



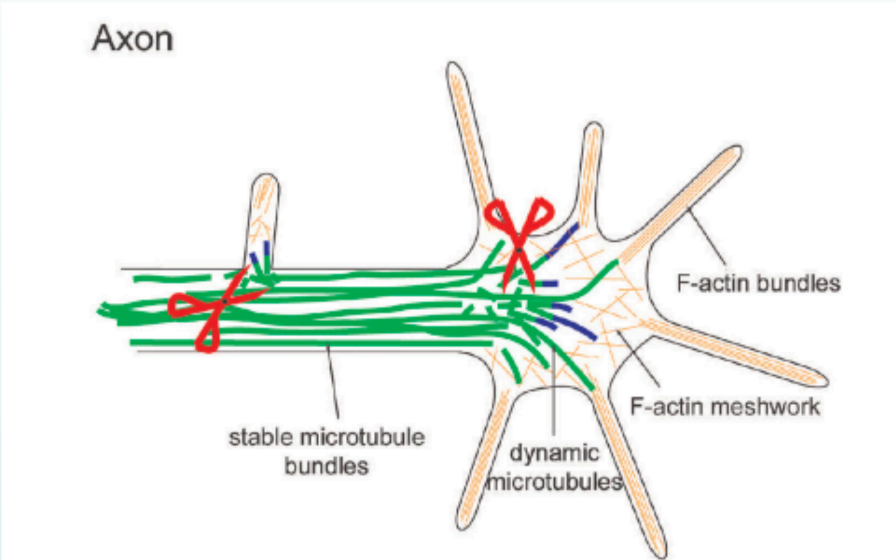
Fidgetin Recap



- Severs GMPCPP microtubules preferentially.
- Depolymerizes like katanin
- Removes protofilaments instead cutting through microtubules (preliminary data)
- Different mechanism of recognition of target?

Microtubule Organization: Stable Microtubules

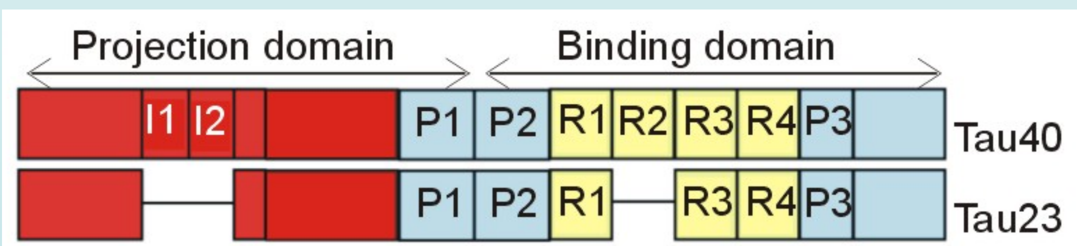
MTs of axon are stabilized by tau



Roll-Mecak and Vale, JCB Comment, 2006

Tau: Axonal microtubule associated protein

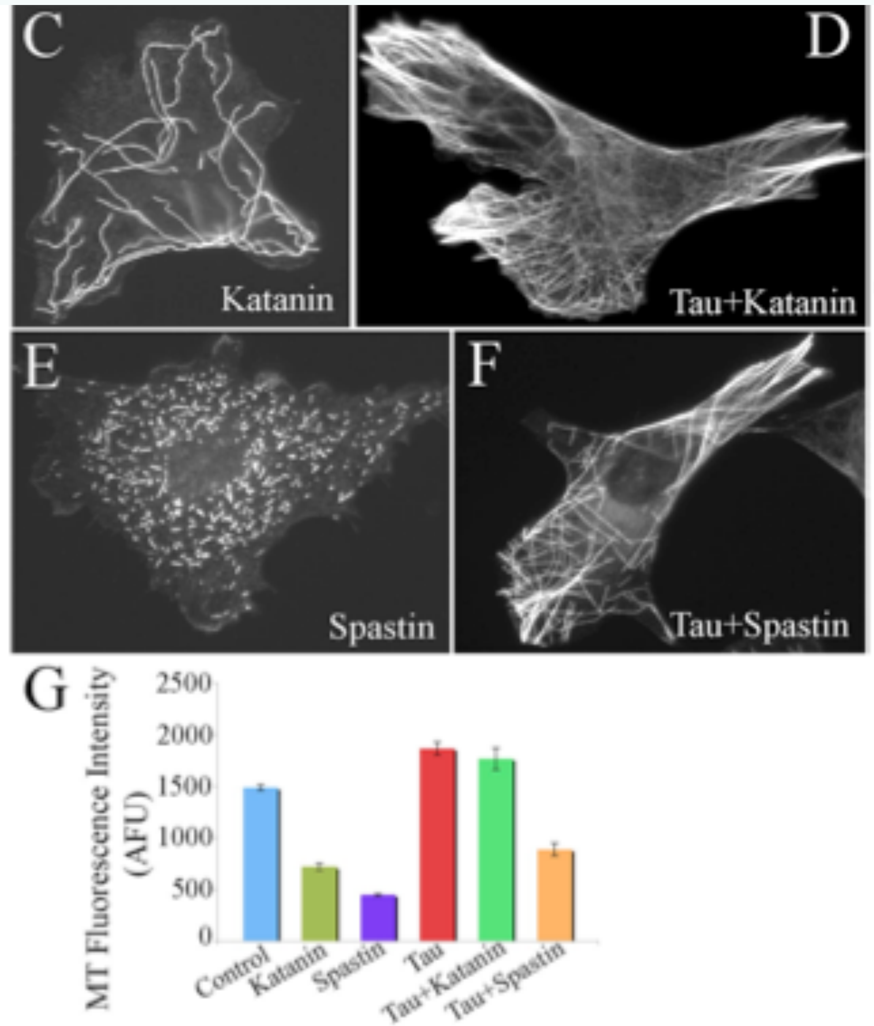
6 isoforms developmentally regulated expression



Controlling Severing with MAPs

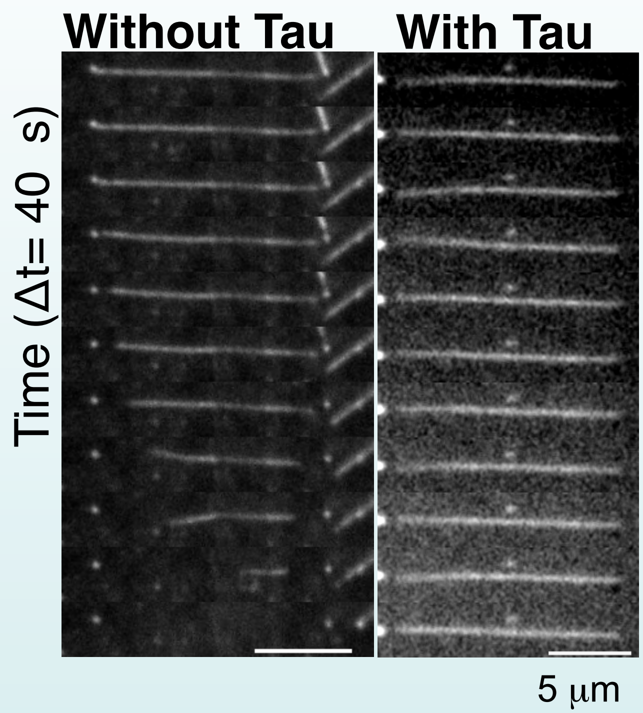
Tau has been shown to inhibit Katanin in cells

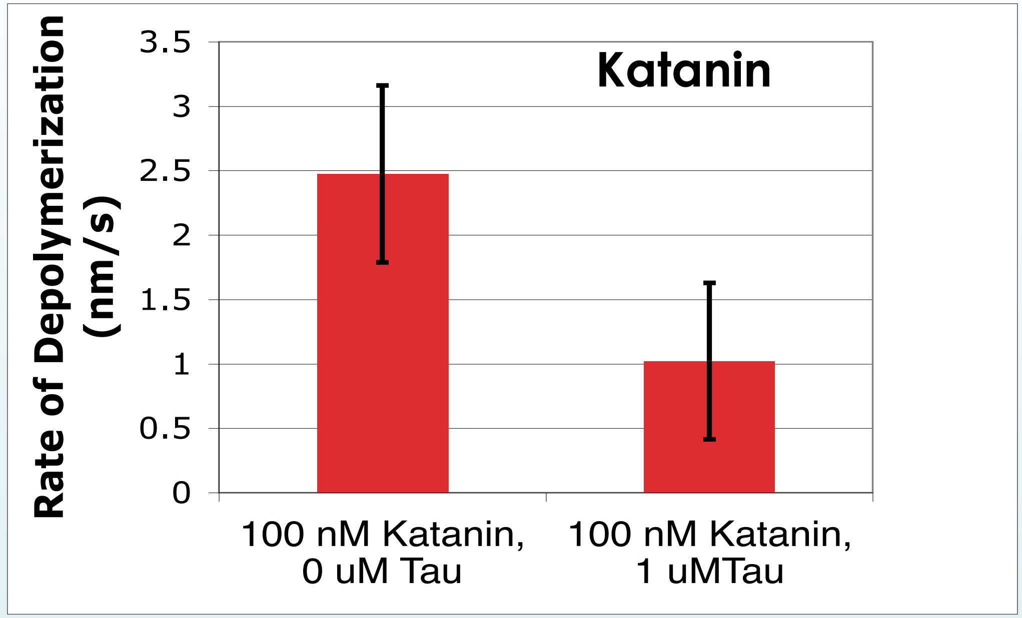
Tau has been shown to have no effect on Spastin in cells



Yu, et al. MBoC (19) 2008

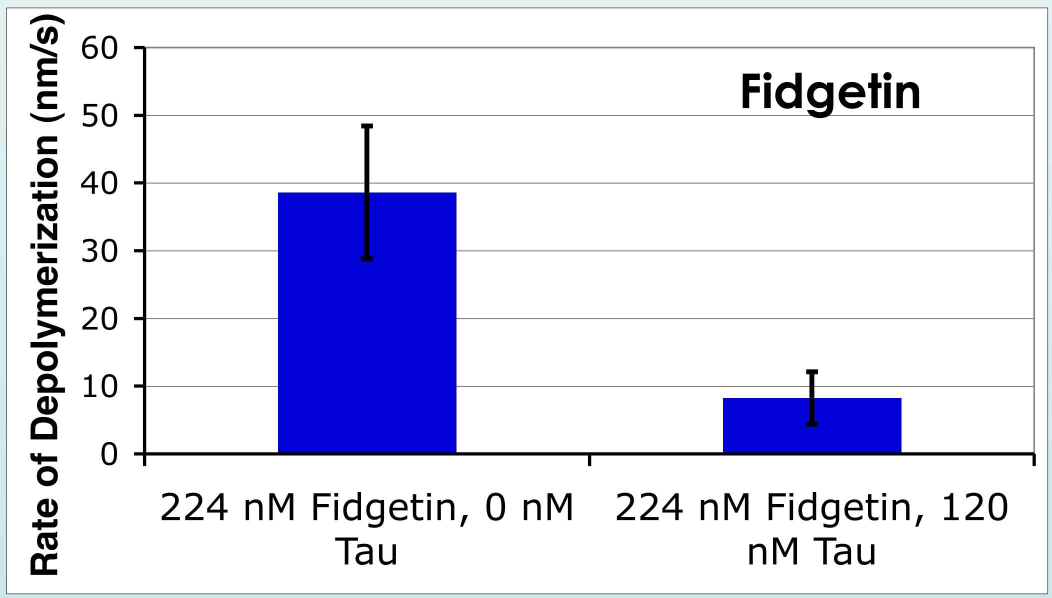
Tau Inhibits Severing and Depolymerization





Tau blocks severing

Inhibits depolymerization

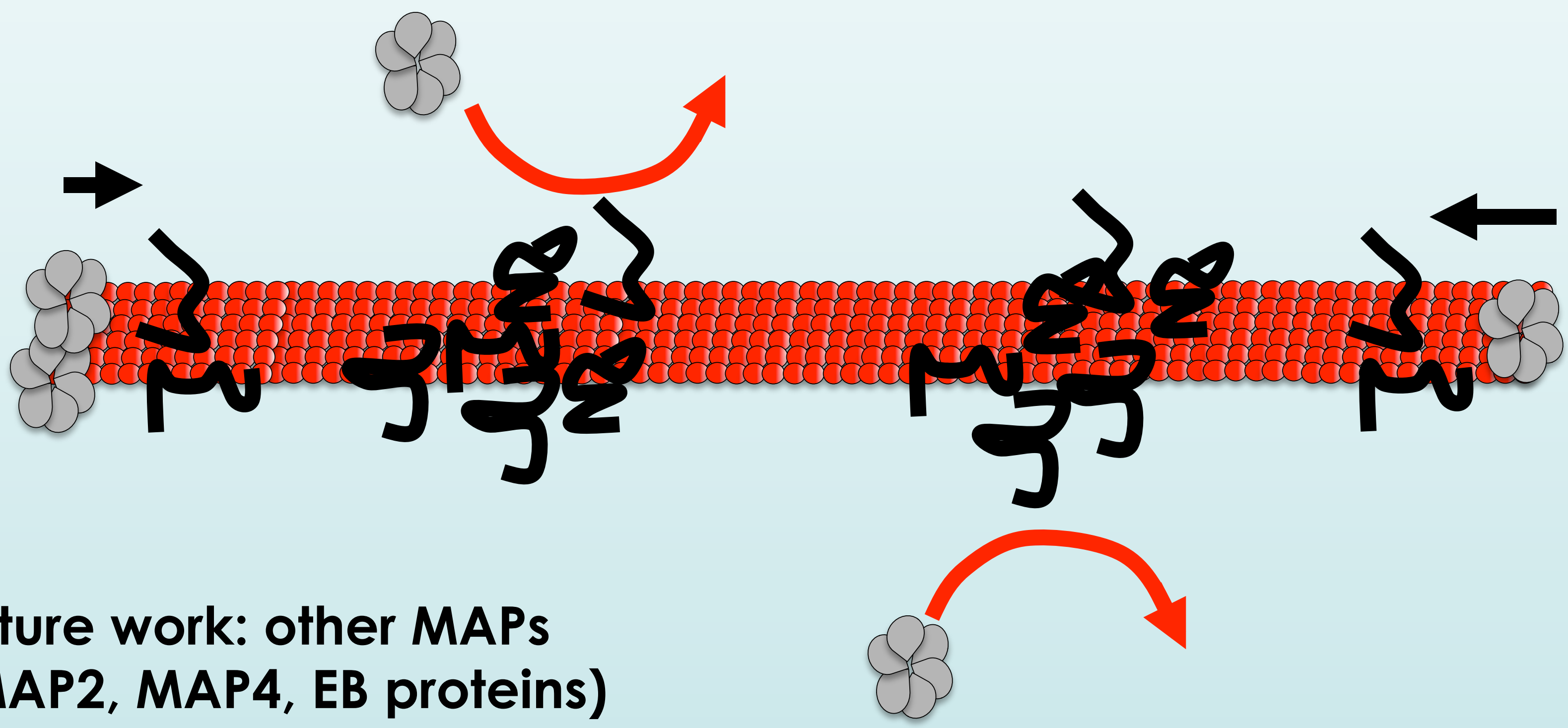


Tau Inhibits Severing Enzymes

Tau binds cooperatively in clumps

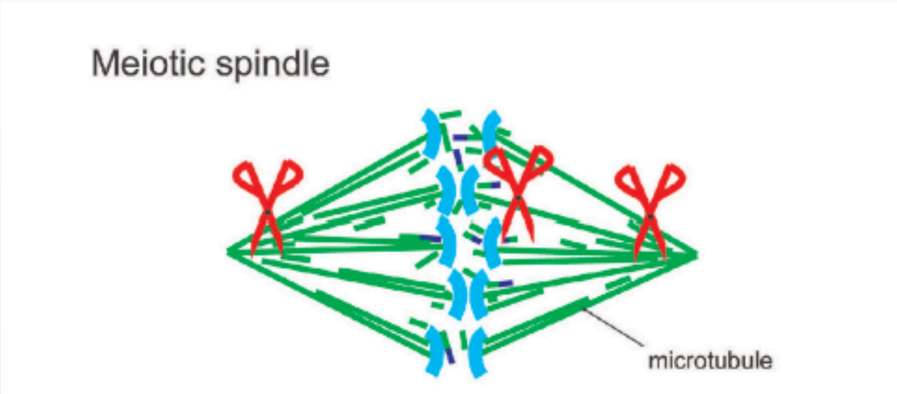
Tau inhibits access of severing enzymes

Tau reduces depolymerization by acting as a steric block?

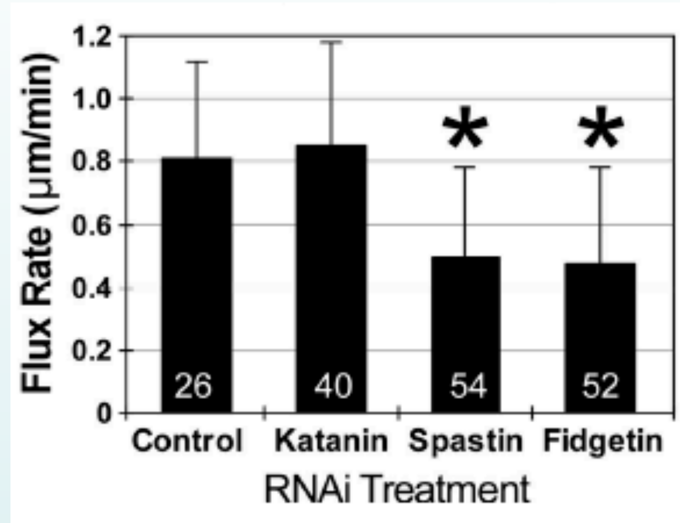


Future work: other MAPs
(MAP2, MAP4, EB proteins)

What about Dynamic Microtubules?



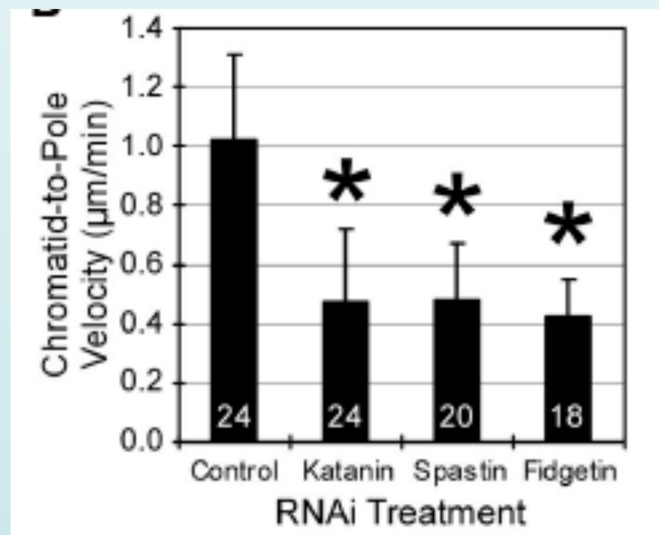
Roll-Mecak and Vale, JCB Comment, 2006



Microtubules of the S2 spindle flux

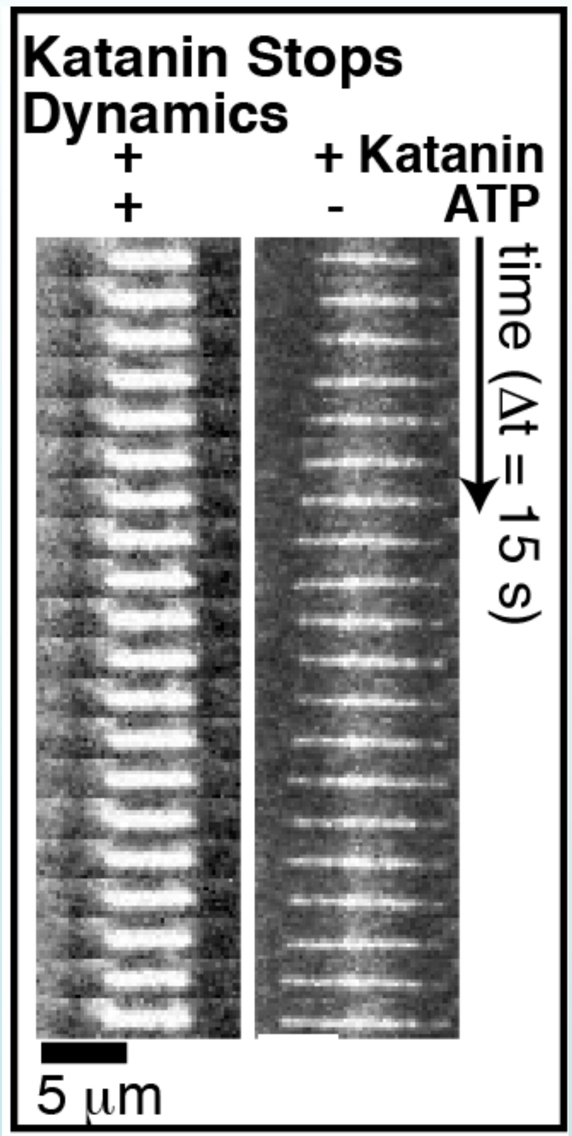
Knocking down spastin and fidgetin reduce flux and inhibit anaphase

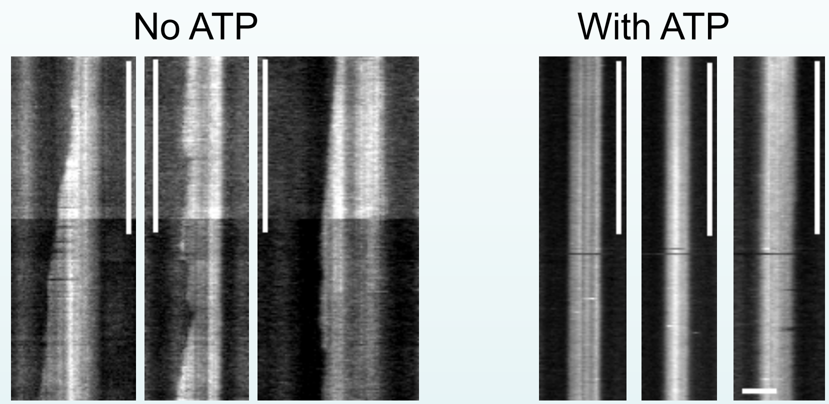
Knocking down katanin inhibits anaphase



Zhang, et al, JCB, 2007

Very Preliminary: Katanin Inhibits MT Dynamics





100 nM Katanin was added to dynamic microtubules (free tubulin + GTP) in the absence or presence of 2 mM ATP.

Without ATP, microtubules displayed normal dynamics.

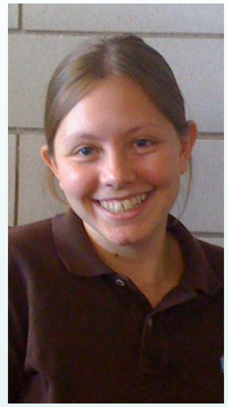
With ATP, microtubules did not grow.

Need to fully explore katanin concentrations (probably more potent on non-stable microtubules).

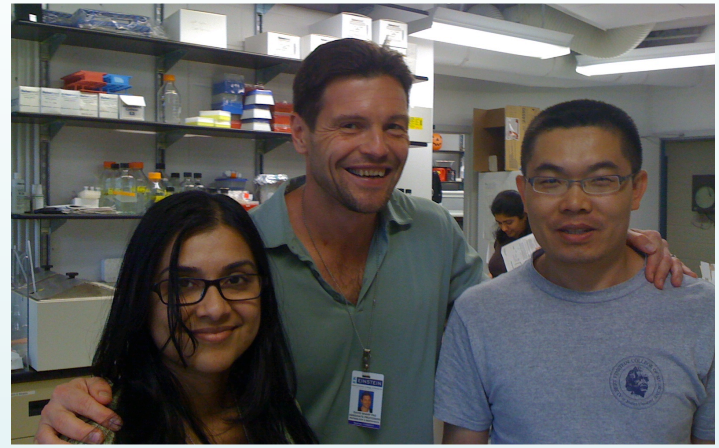
Thanks…

Daniel Diaz (postdoc)

Margaret Morelli (undergrad)



Megan Bailey (rotation student)



Suranjana Mukherjee, David Sharp, Ray Zhang (Albert Einstein)

Hernando Sosa

This work supported by March of Dimes Basil O' Connor Starter Award

This afternoon: poster on new measurements of microtubule mechanics. Please stop by!

Other funding from NSF and Research Corp