Neurite formation & neuronal polarization

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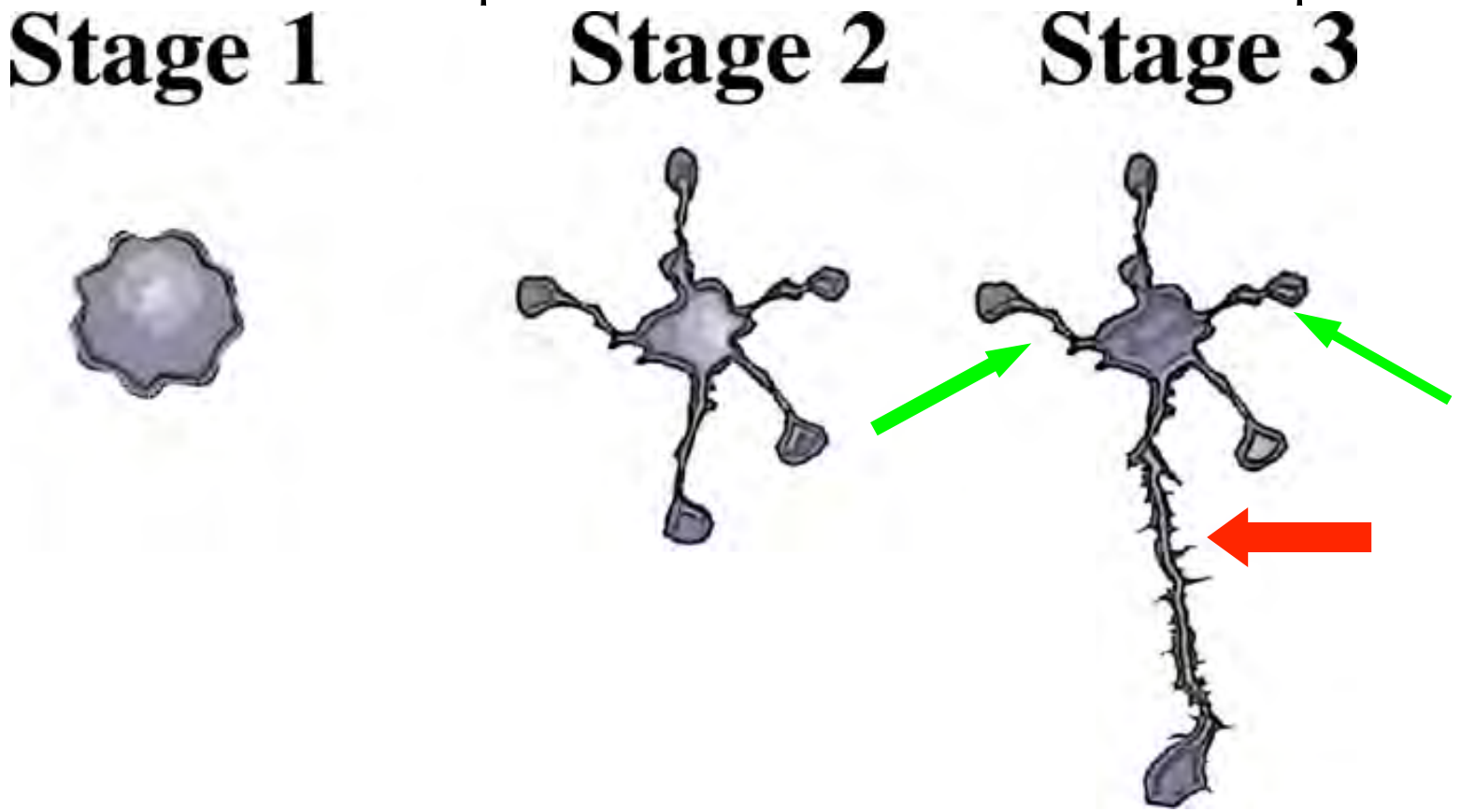
Chapter 16; The Cytoskeleton; **Molecular Biology of the Cell,** Alberts et al.

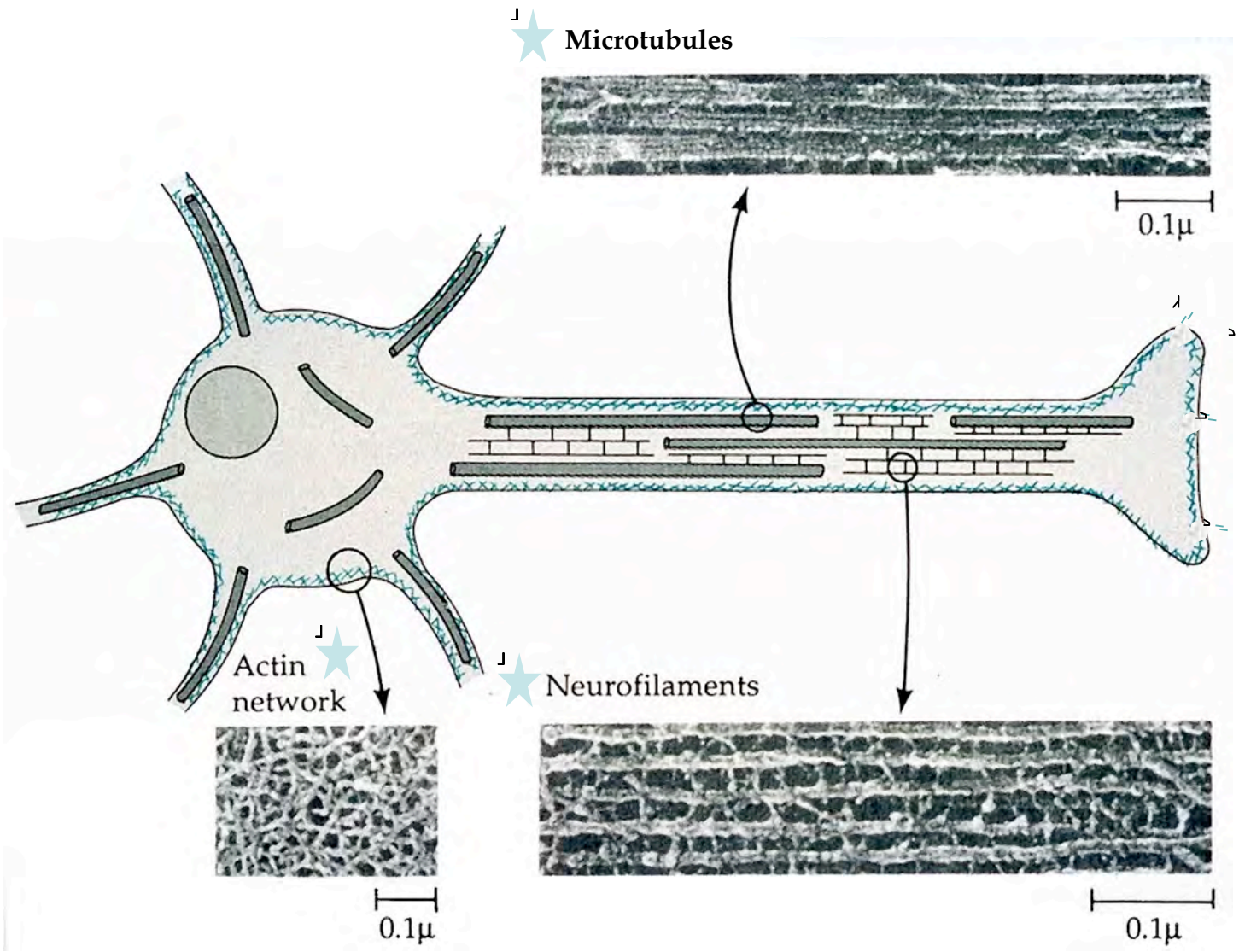
•An immature neuron in cell culture first sprouts thin processes, called neurites (stage 2).

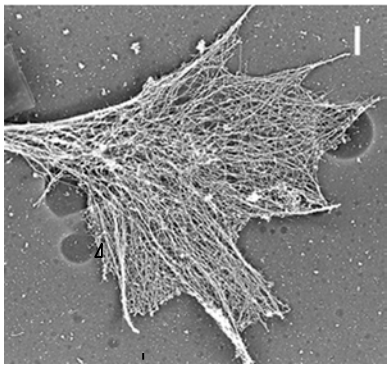
•After a day or so, one neurite accelerates its growth and becomes the axon. The other neurites become dendrites (stage 3).

•Axons are the long wires that interconnect neurons, while dendrites are shorter processes that receive axonal inputs.

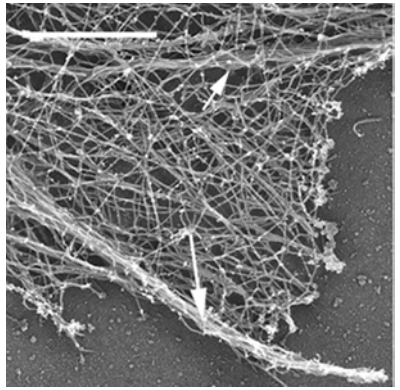


The cytoskeletal components of neurons have characteristic distributions and associations

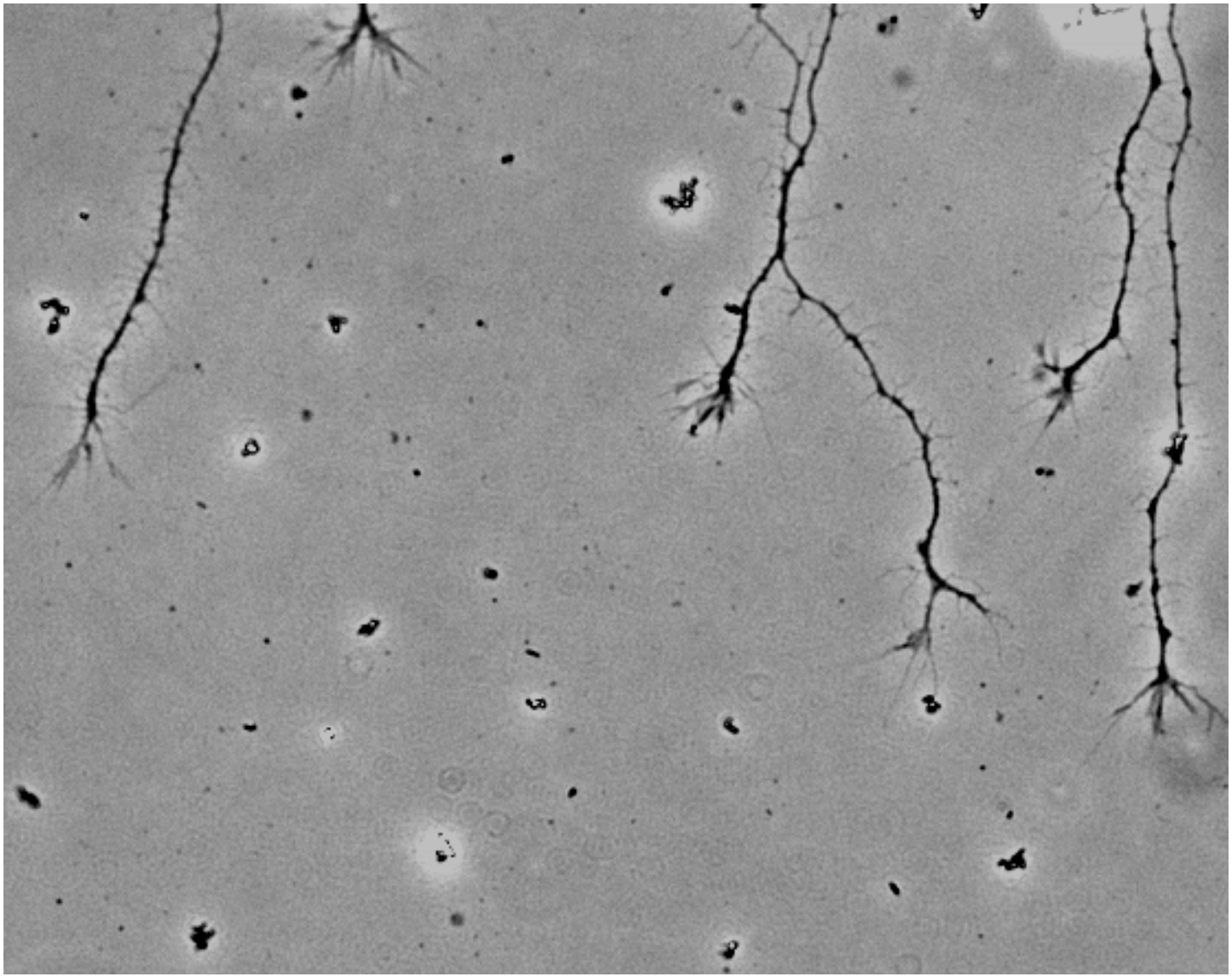




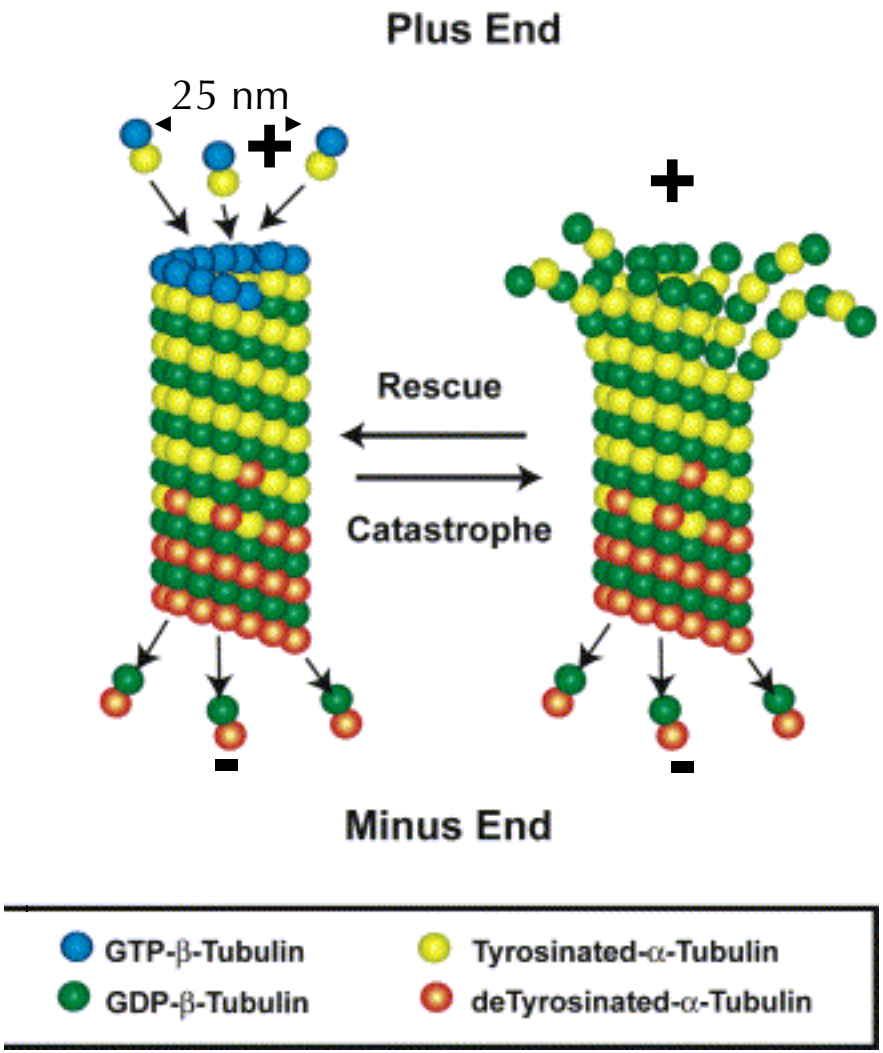
actin



- Neurite elongation is driven by the advance of microtubules by way of polymerization and forward sliding of microtubules.
- Actin-based motility at the distal neurite terminal (growth cone) pulls the neurite forward and sideways.



Microtubules support cell shape and elongation



- MTs are polymers of tubulin dimers. A MT's diameter is 25 nm. Individual microtubules resist compression.

- Polymerization requires GTP bound to tubulin dimers

- have intrinsic polarity - important in transport

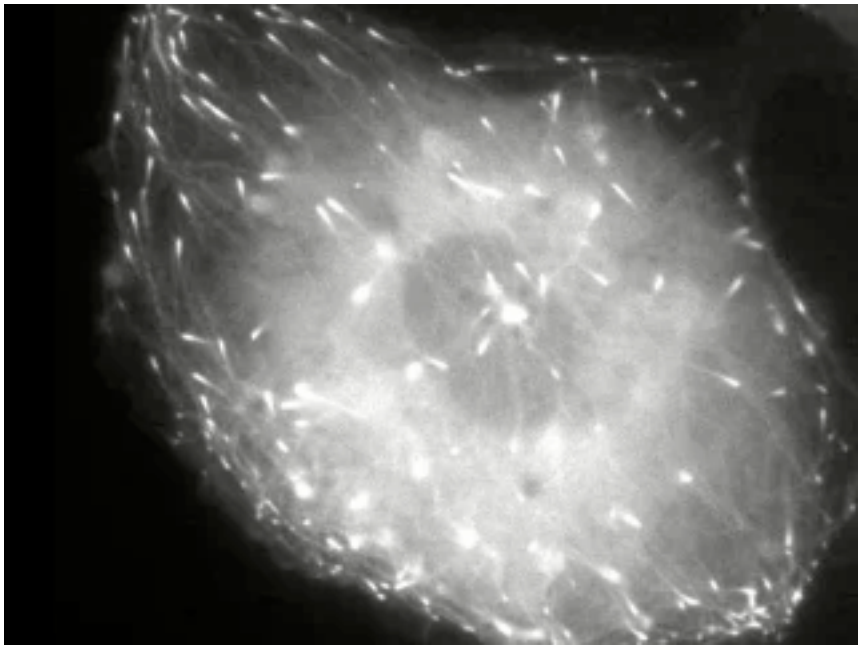
- plus ends alternate between states of growing, shrinking (catastrophe) and resting.

Microtubules alternate between growing and shrinking plus ends: dynamic instability

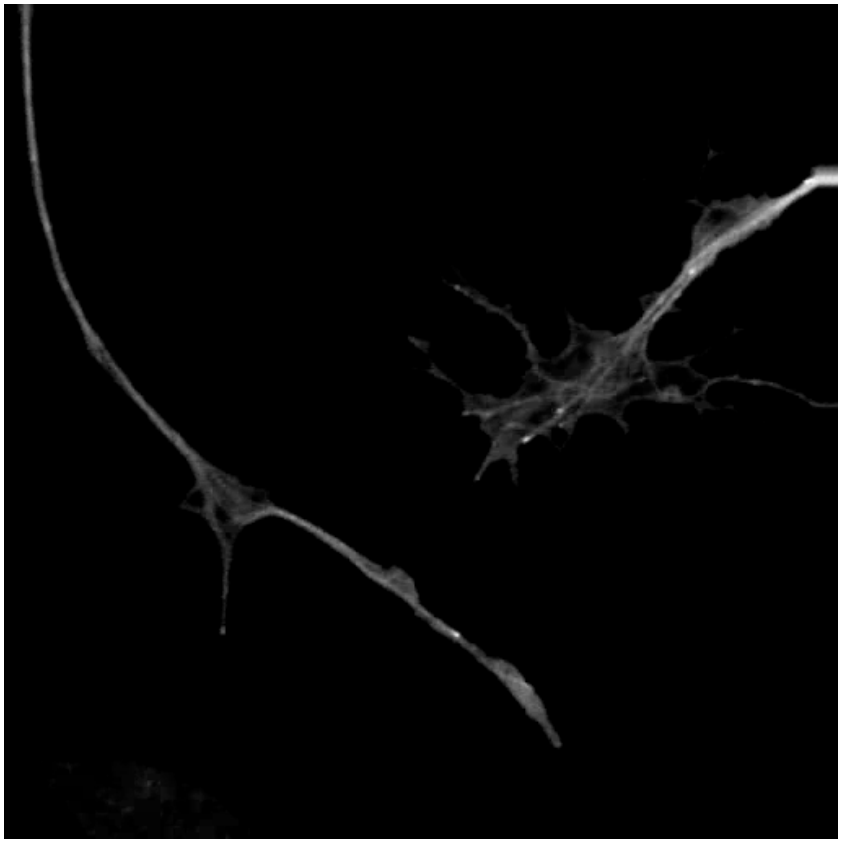
Imaging of microtubule dynamics in live cells

EB3-GFP marks MT + ends

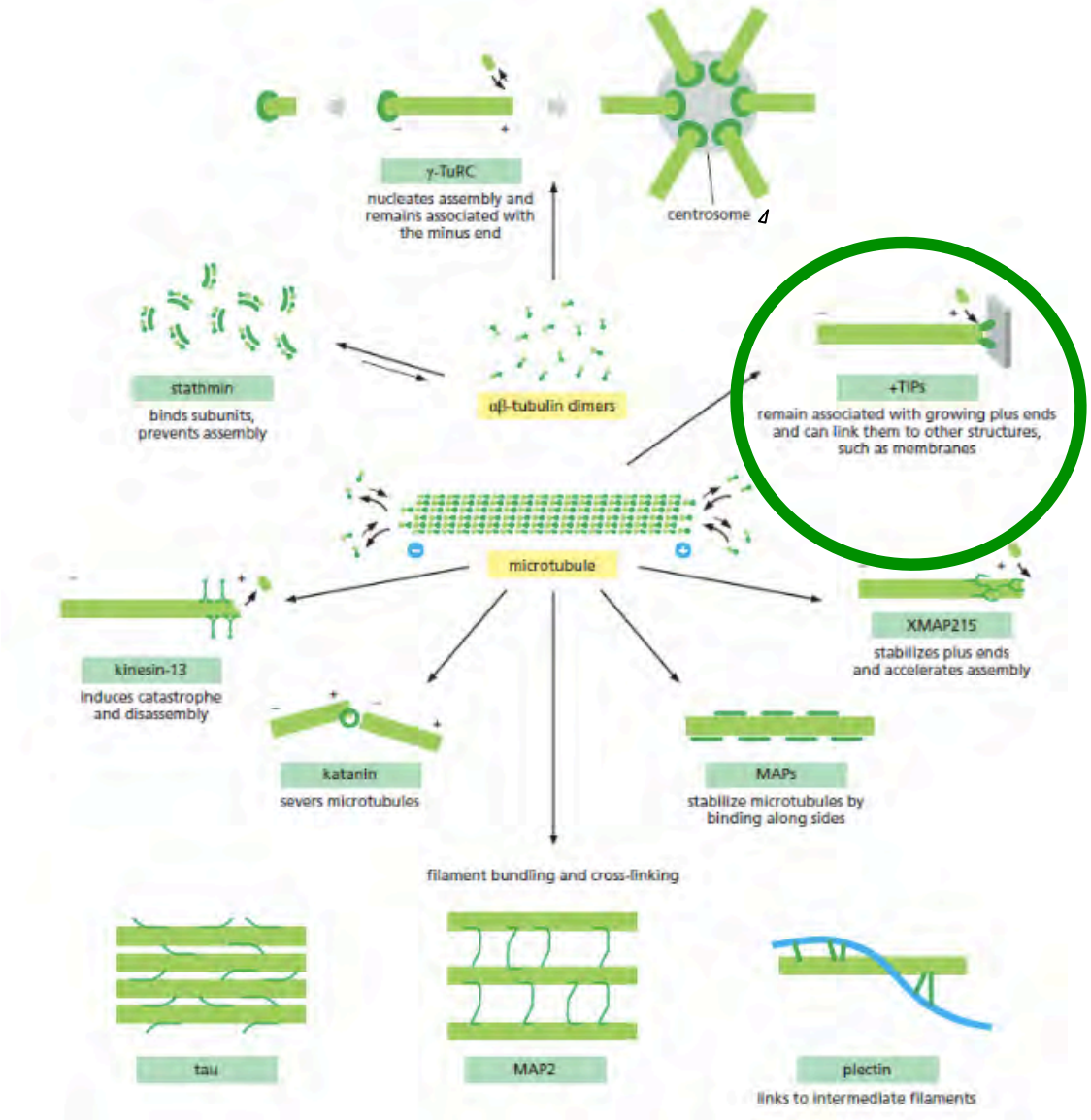
microtubules in fibroblast



microtubules in a neuron

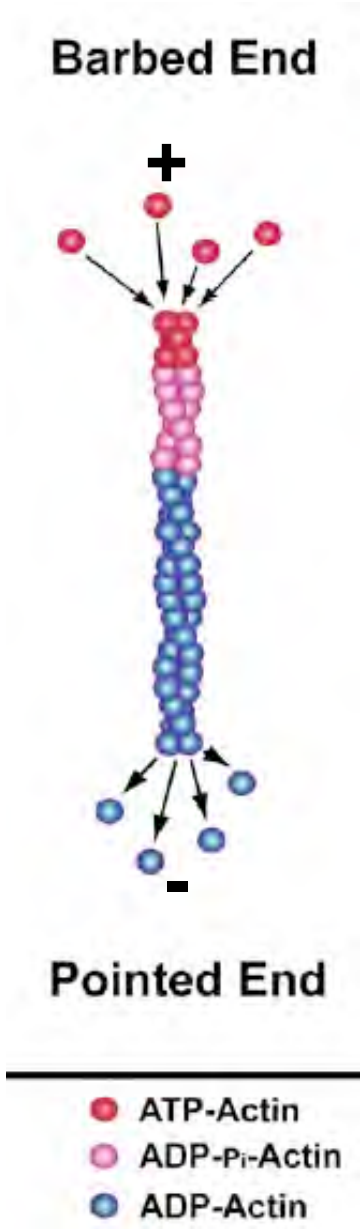


•In a neuron microtubule initiation, polymerization, stability and interactions are regulated by **microtubule-associate proteins**, collectively called MAPs.

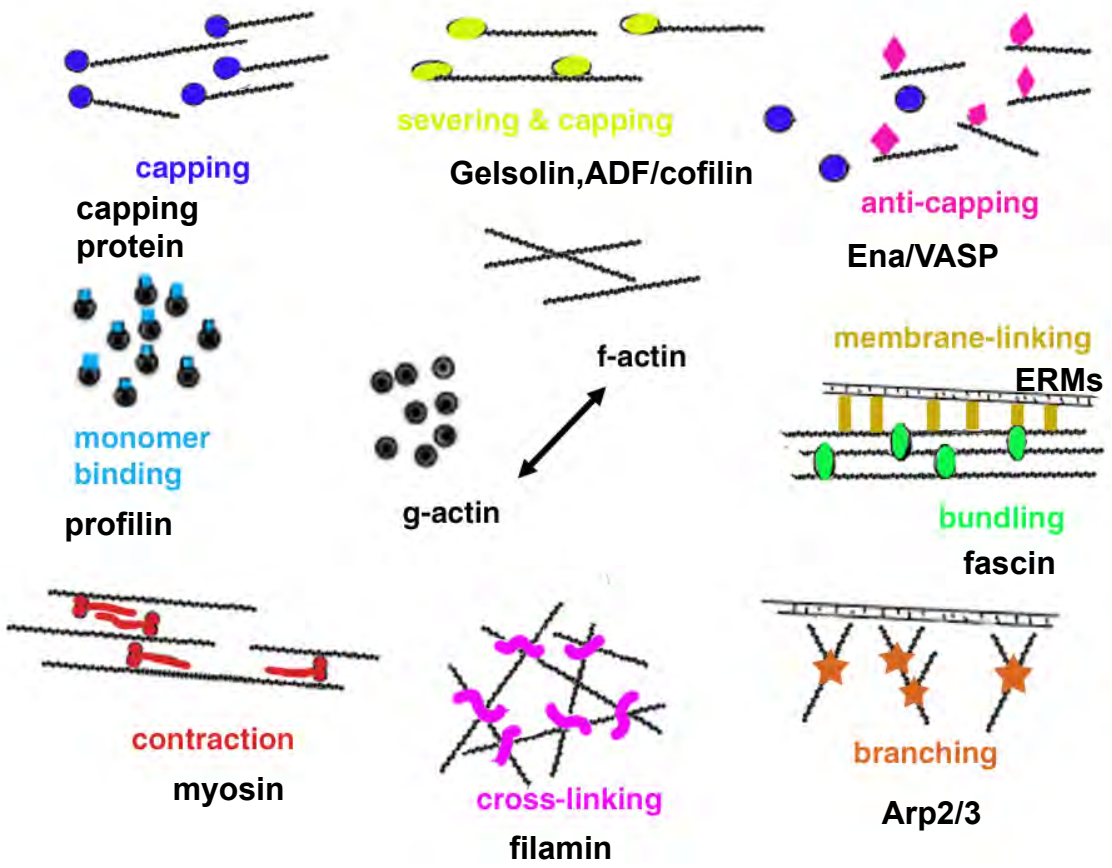


Actin filaments support the cell cortex and produce dynamic cortical motility

Filamentous actin (F-Actin) is a dynamic polymer of actin monomers



Actin-binding proteins regulate actin polymerization and organization



- F-actin organization, dynamics and functions depend on:
  - localization
  - relative concentrations
  - activitity levels of:

Actin-binding proteins

F-actin dynamics at the front of a migrating cell

Motor proteins use energy from ATP hydrolysis to create mechanical energy that moves cargo or exerts tension (a pulling force).

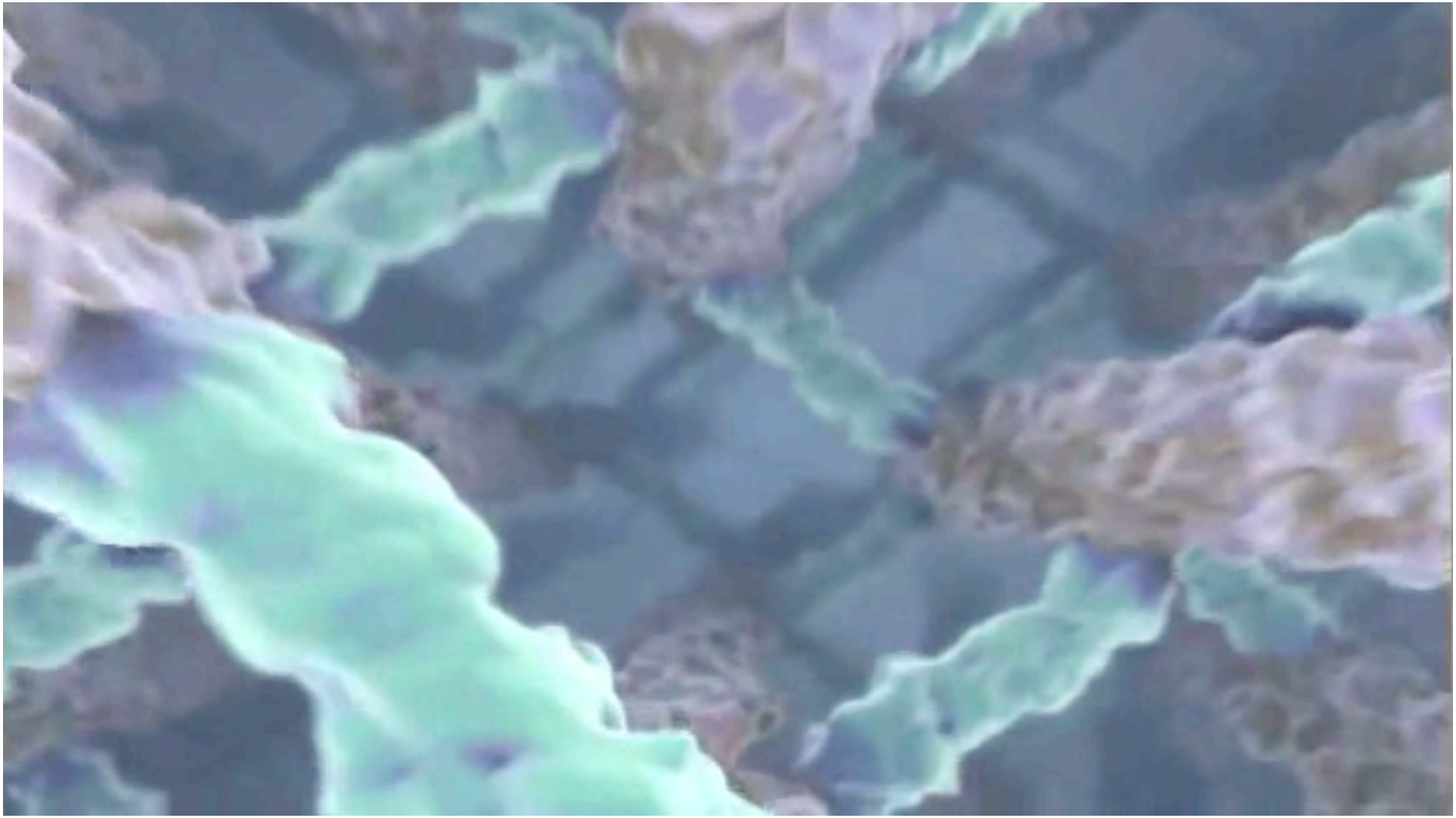
1. A tail domain at one end attaches to a cargo or structure.
2. A mobile head domain at the other end attaches to a MT or AF and hydrolyzes ATP to power its walking along the filament.
3. An individual motor protein moves in only one direction along a MT or AF.
4. The movement generated by a motor protein <u>depends on the relative resistance or anchorage</u> of the filament vs. the cargo or attached structure.
5. If both the protein filament and the cargo resist movement, tension is generated and maintained while the motor is attached to the cargo and the filament.

Motor proteins move cargo along MT and AF

Filament	Motor	Direction motor walks	Cargo	neuronal function?
Actin filament	myosin II	toward + end	actin filaments?	migration, retrograde flow & retraction
Actin filament	myosin V	toward + end	vesicles	
Actin filament	myosin VI	toward - end	vesicles	
Actin filament	myosin X	toward + end	integrin?	filopodia adhesion?
Microtubule	dynein	toward - end	vesicles, MT?	retrograde axonal transport, MT transport?
Microtubule	kinesin(s)	toward + end	vesicles	RNA & vesicle transport neuronal polarity
Microtubule	CHO1/MKLP1	toward + end	MTs (-end distal)	dendritic MT polarity

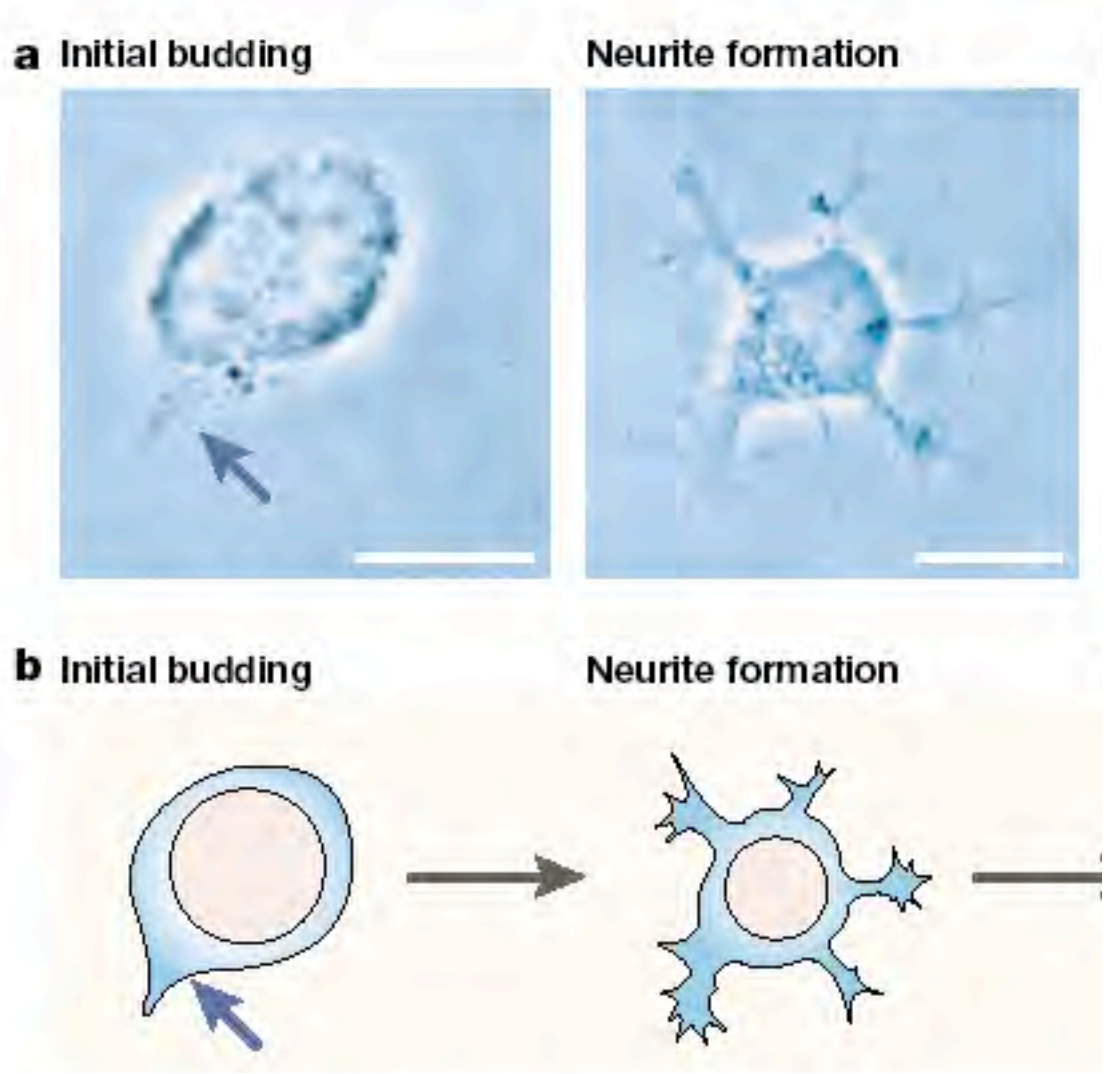
View an amazing animation of actin filaments, microtubules, motor molecules, cell adhesion, cell secretion, cell movement, called The Inner Life of the Cell at https://www.youtube.com/watch?v=zrXykvorybo

The Inner Life of the Cell

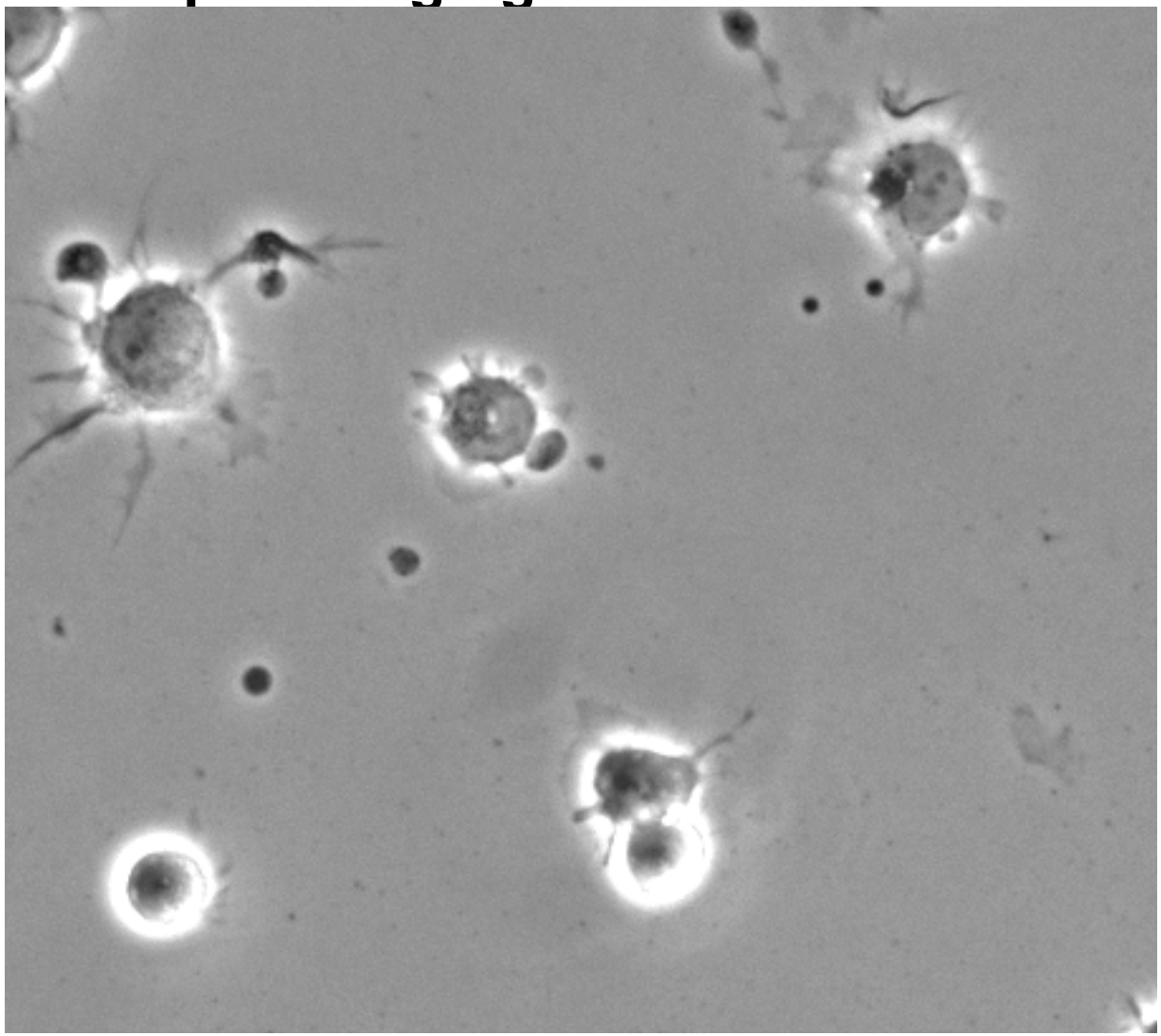


https://drive.google.com/file/d/0B_eeKdmafJUYMkRYbGcxZjFqVk0/view

•Neurite formation begins with a filopodium or bud that sprouts from the cell body.
• Several neurites can sprout from a neuron.



Time lapse imaging of neurite initiation

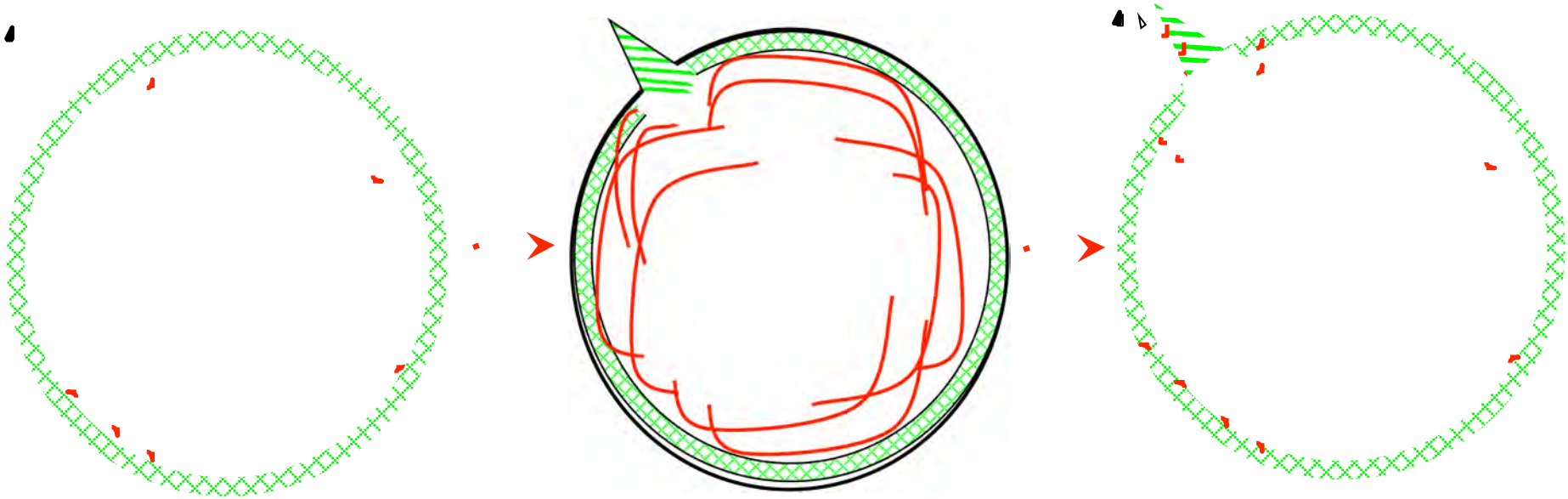


- **What triggers the transition from a filopodium to a neurite?**
- **A critical step in neurite initiation is the entry of a microtubule(s) into a filopodial bud.**

Symmetry of perikaryon

A filopodium extends out.

The symmetry is broken! Microtubules enter the bud.



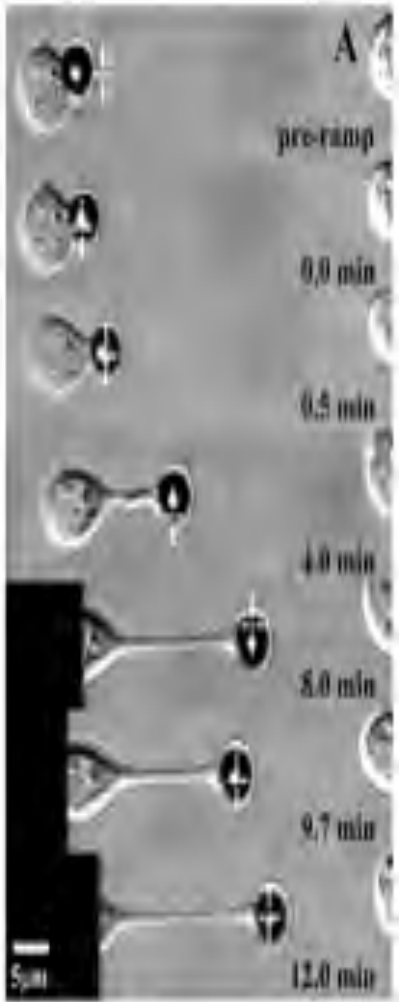
Cortical actomyosin tensions

Internal and extrinsic forces contribute to moving microtubules into a filopodium

Neurite formation can be initiated by a localized tension that pulls out the cell cortex

- **Tension exerted in an adherent filopodium may pull a microtubule end into the base of the filopodium**.

- Experiments in the lab of Dr. David Odde (BME Dept) showed that extrinsic manipulations to exert tension (pulling) on the neuronal surface of a cultured neurons can elicit the initiation of a neurite from the point of tension.



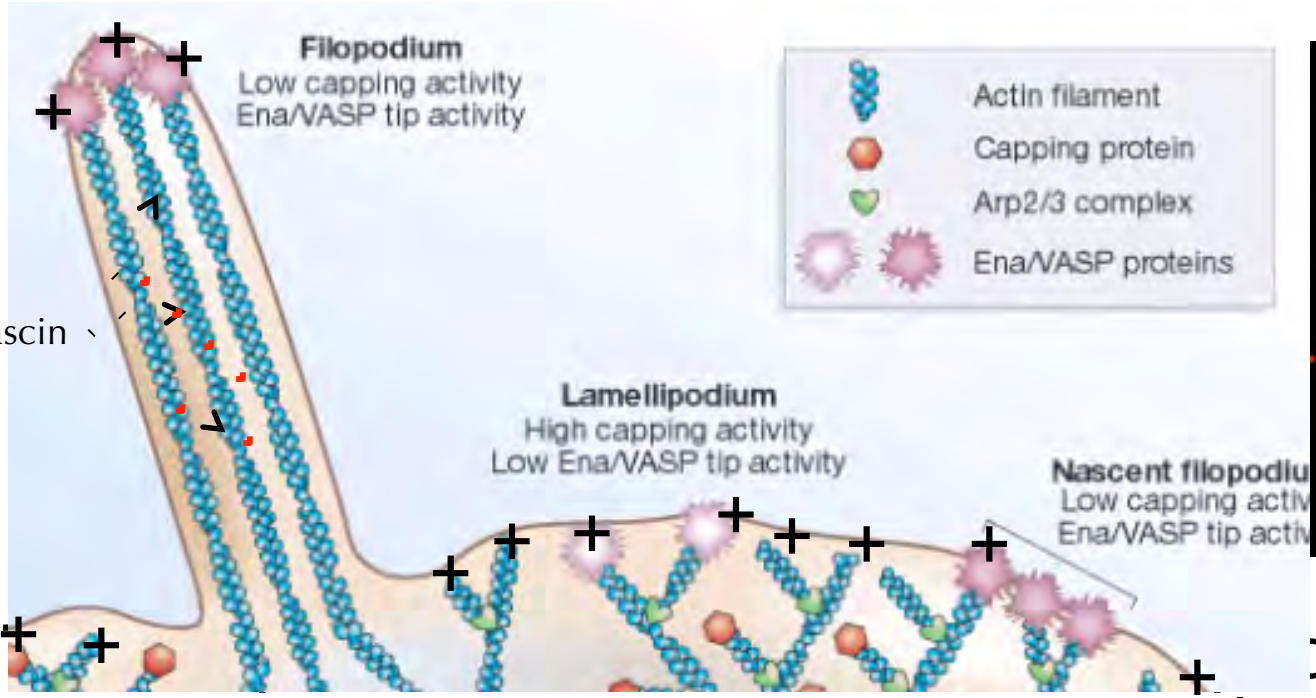
Fass & Odde (2003) Biophys 85(1):623-36

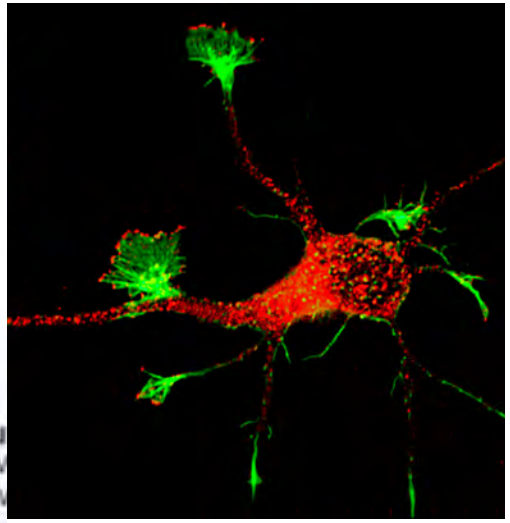
Filopodial protrusion and tension can induce neurite formation

•Ena/VASP proteins are ABPs enriched at filopodial tips.

•Ena/VASP proteins promote prolonged actin polymerization and formation of F-actin bundles.

•Neurons in mice with no Ena/VASP function are deficient in axon formation.

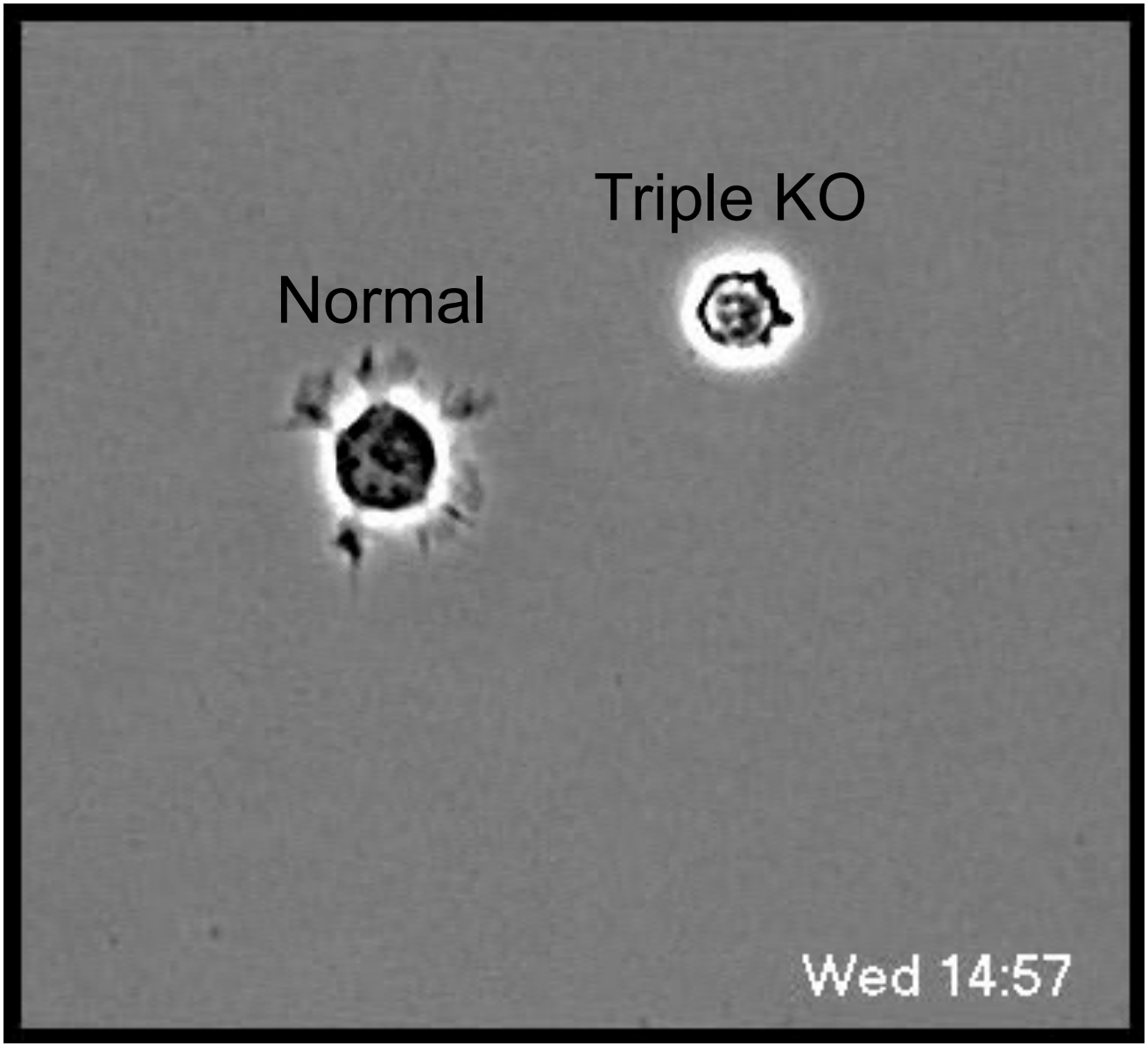




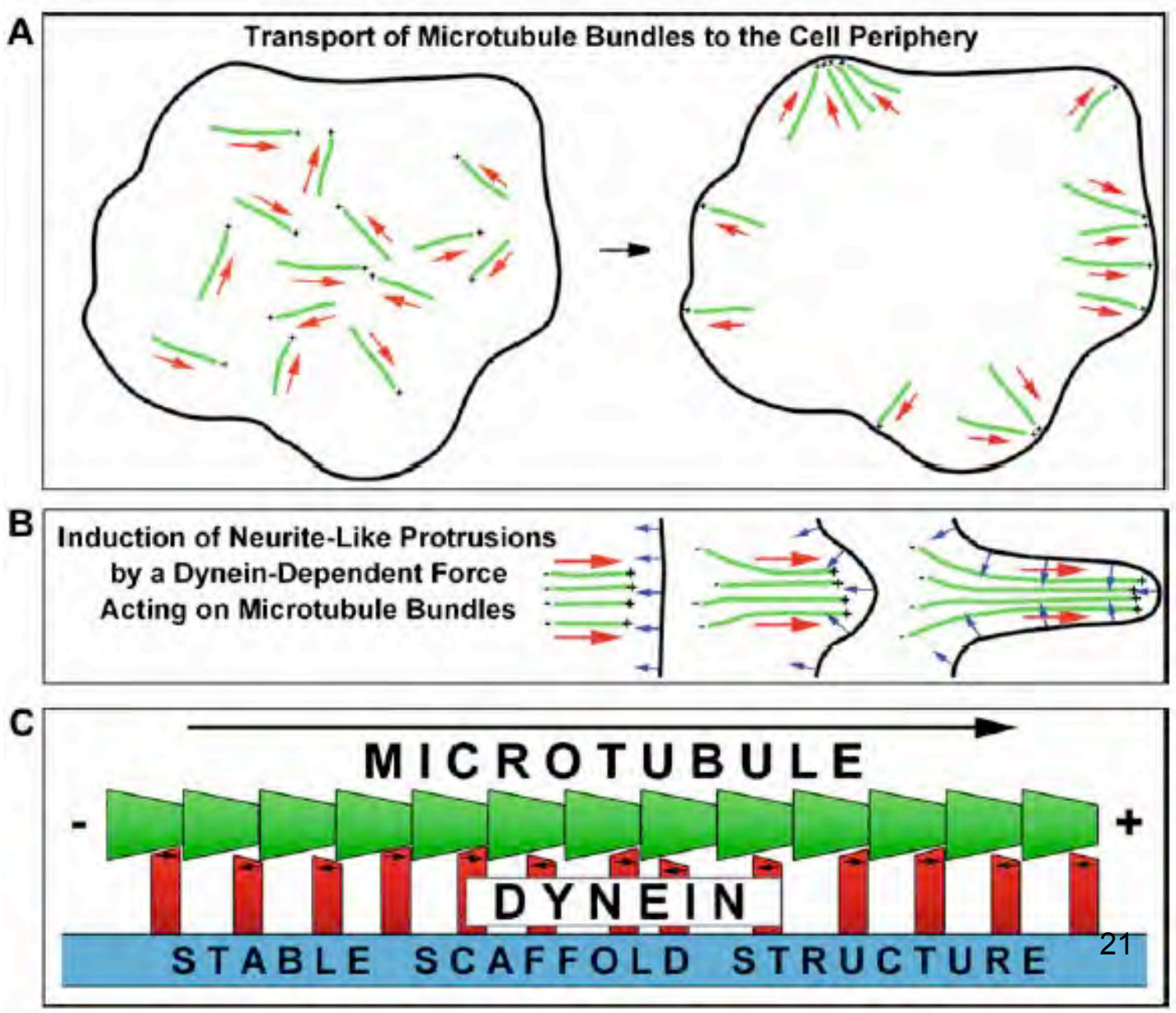
dia may be related to locally "pulling out" of the cell cortex.

Dent et al. (2007) Nature Cell Biol 9(12):1347.

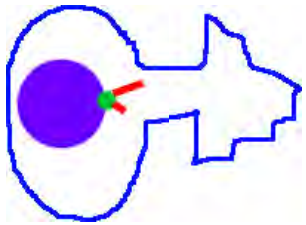
Neurite initiation fails in an Ena/VASP triple KO neuron



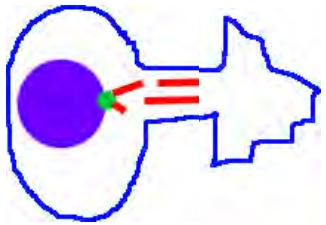
Dynein, a MT motor that walks toward MT – ends, can slide short MTs with their plus ends forward, pushing them outward.



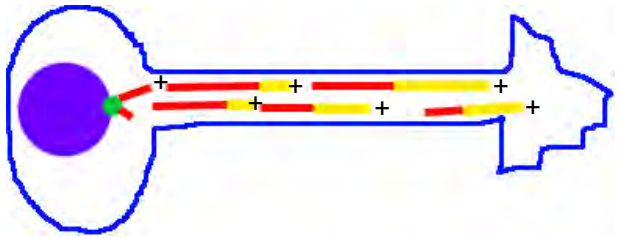
Neurite growth is a continued process of moving and polymerizing microtubules onward into the neurite tip.



MT nucleation at centrosome. Dynamic F-actin motility at neurite tip.

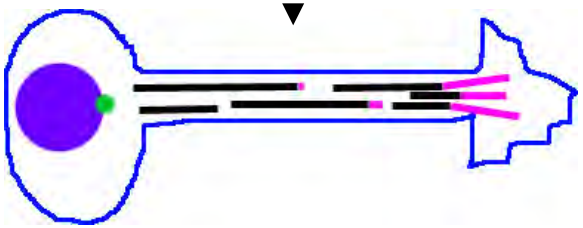


Release and transport of short MTs into the neurite by dynein motors



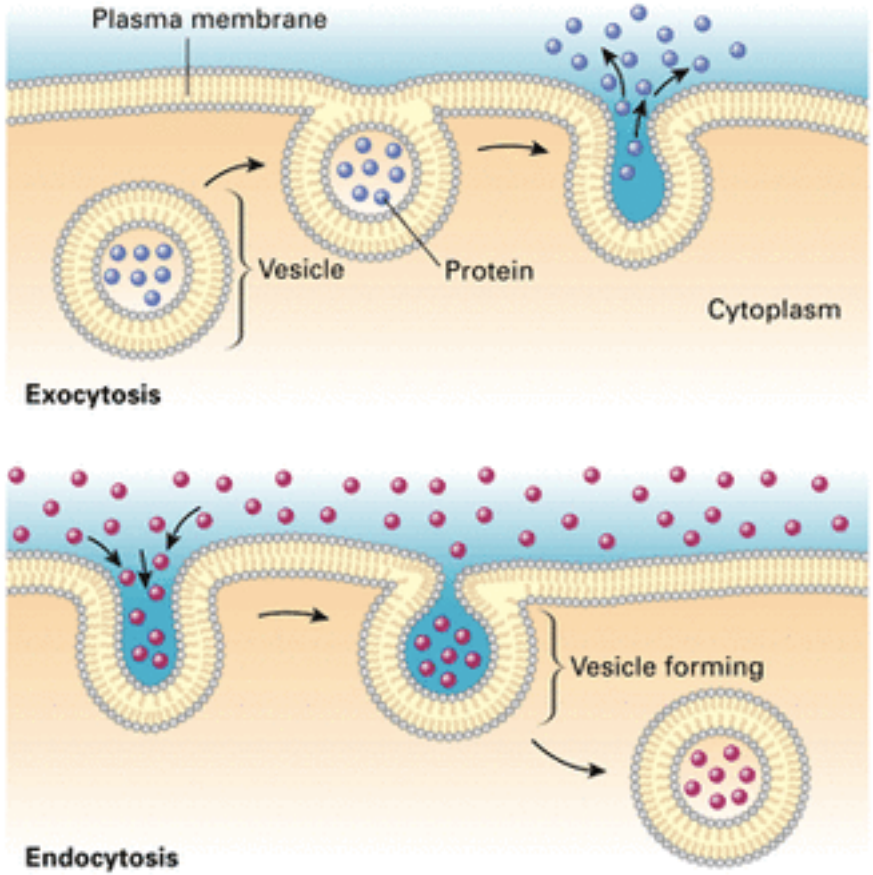
Tubulin subunits are transported and add to existing MTs. MTs become too long to be moved. All MT plus ends point distal.

time

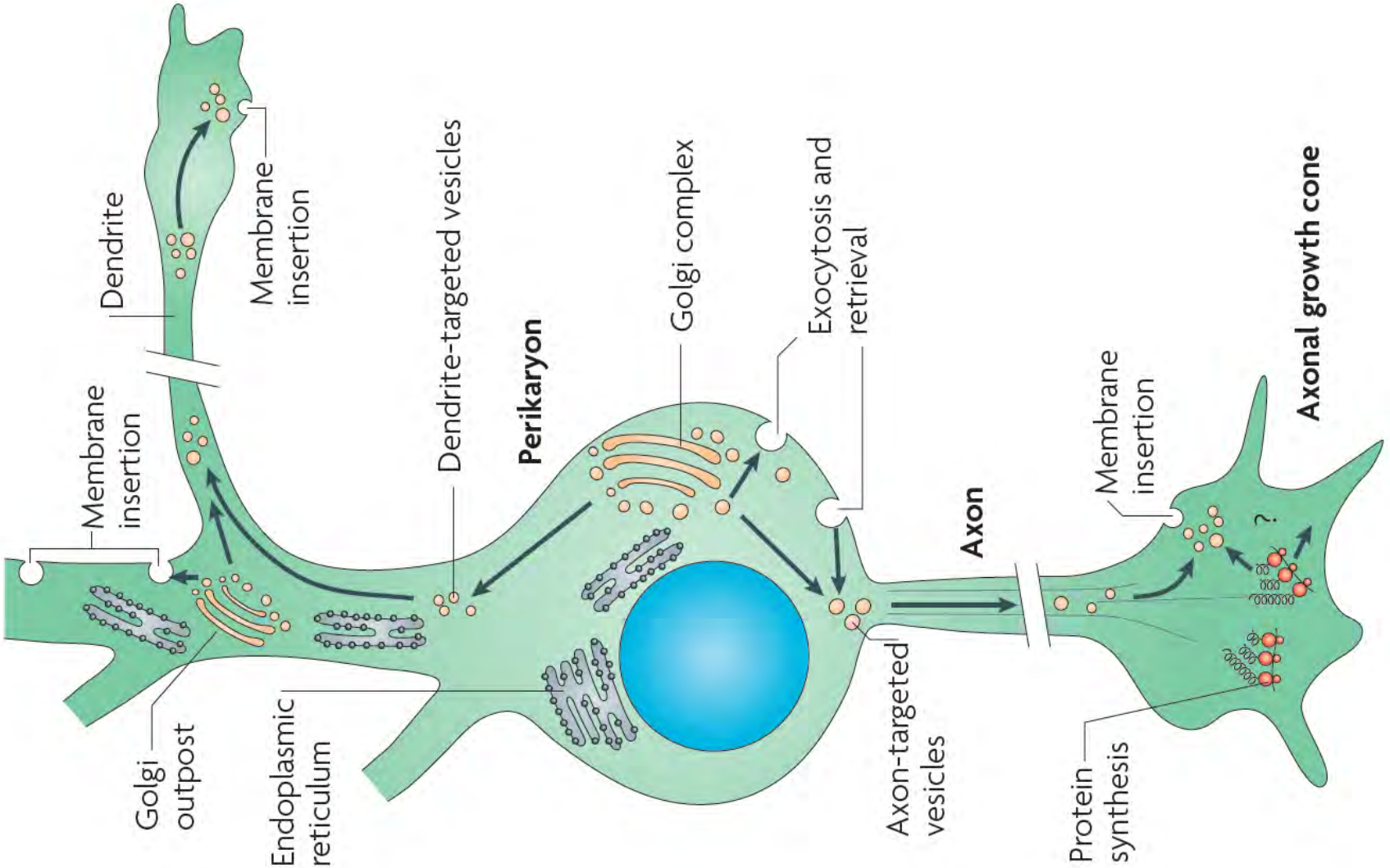


Binding of MAPs produces a gradient of MT stability in neurite

The neurite plasma membrane expands by exocytotic addition of membrane to the neurite surface.

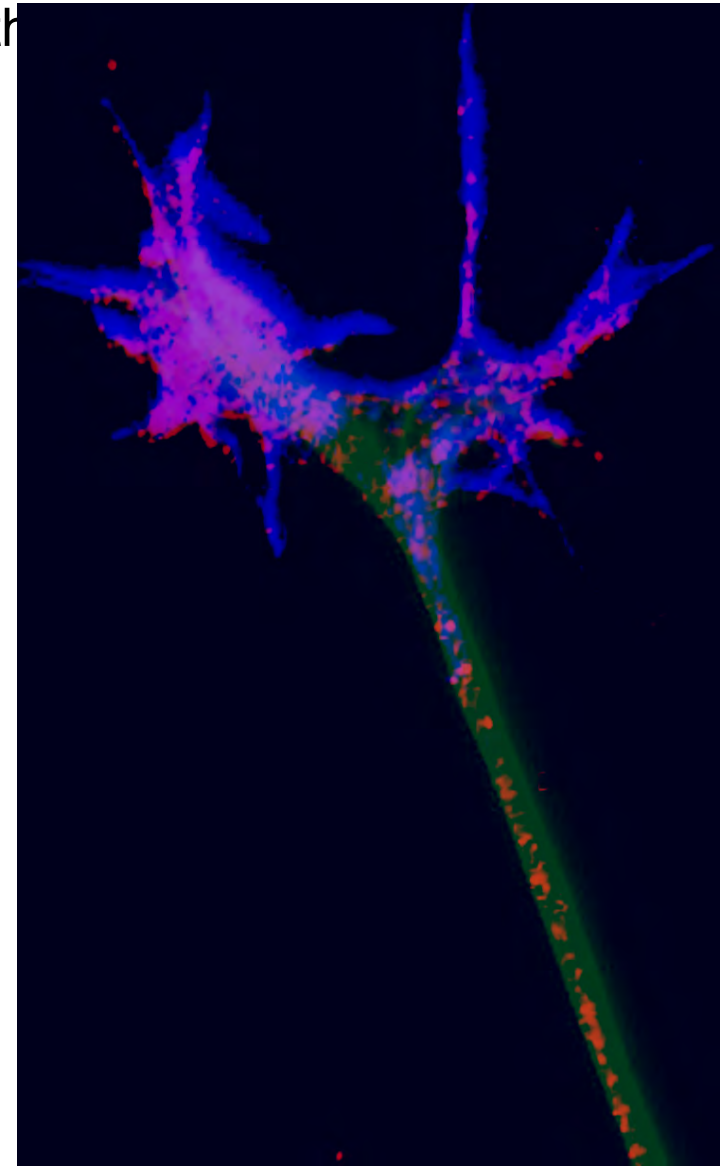


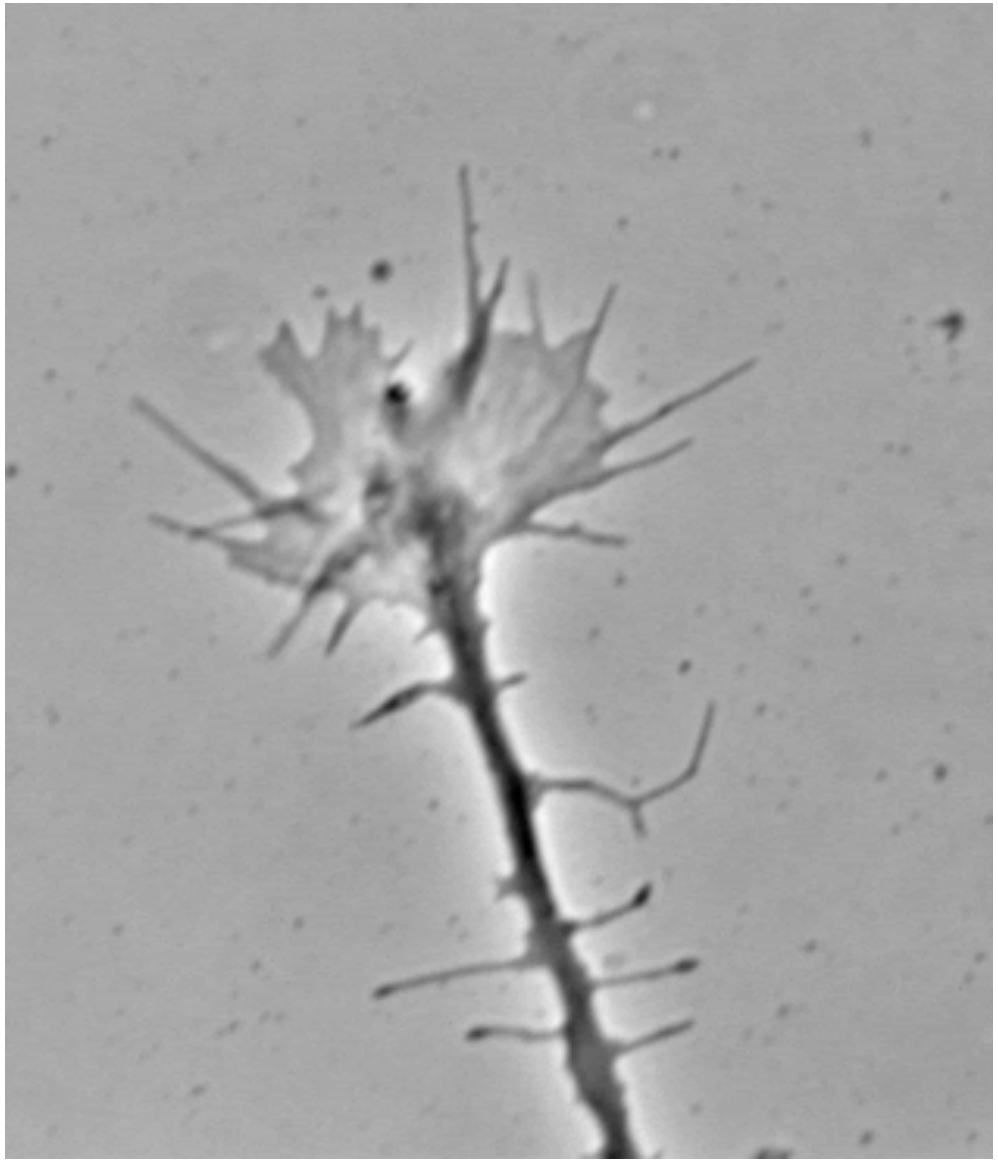
- The growth cone surface expands by exocytosis.
- Plasma membrane can also be removed by endocytosis.
- Specific membrane components can be added or removed.
- Exocytosis and endocytosis are most dynamic at growth cone.



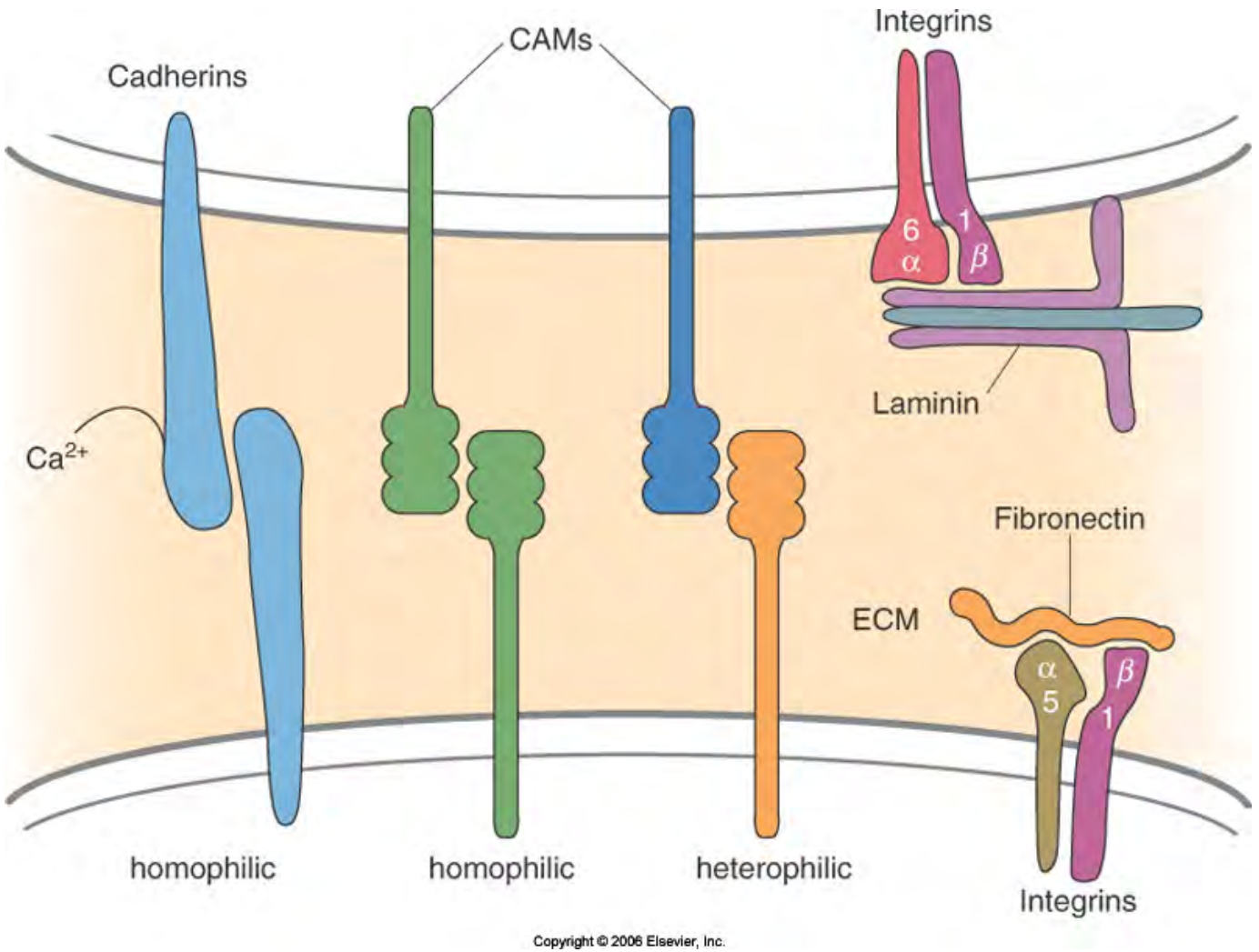
•The actin filament network beneath the plasma membrane includes myosin II.

•This creates a compressive force in the cortex that could collapse and retract th





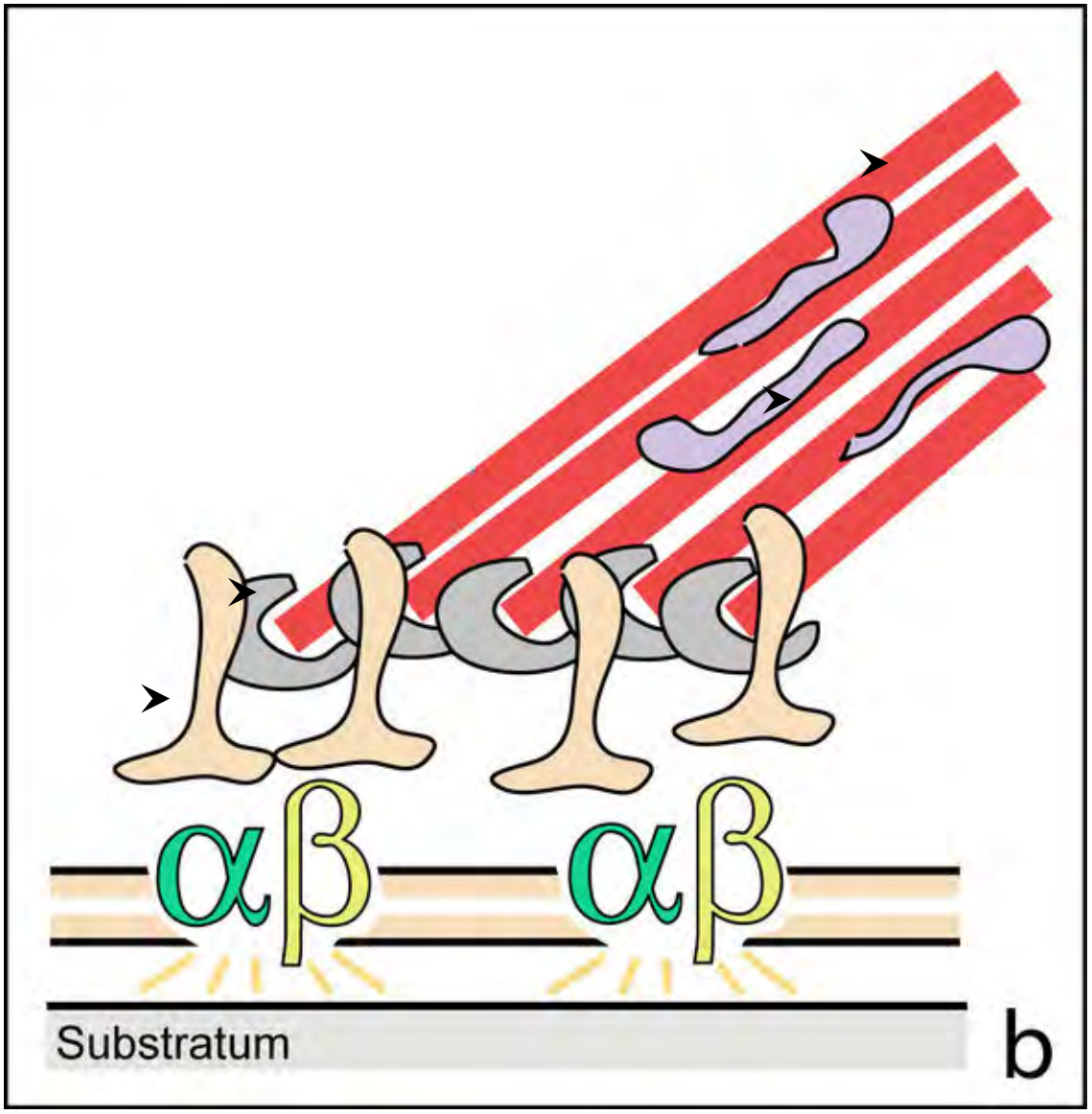
•Neuron-substrate adhesion is mediated by three types of adhesion receptors on neuronal surfaces.



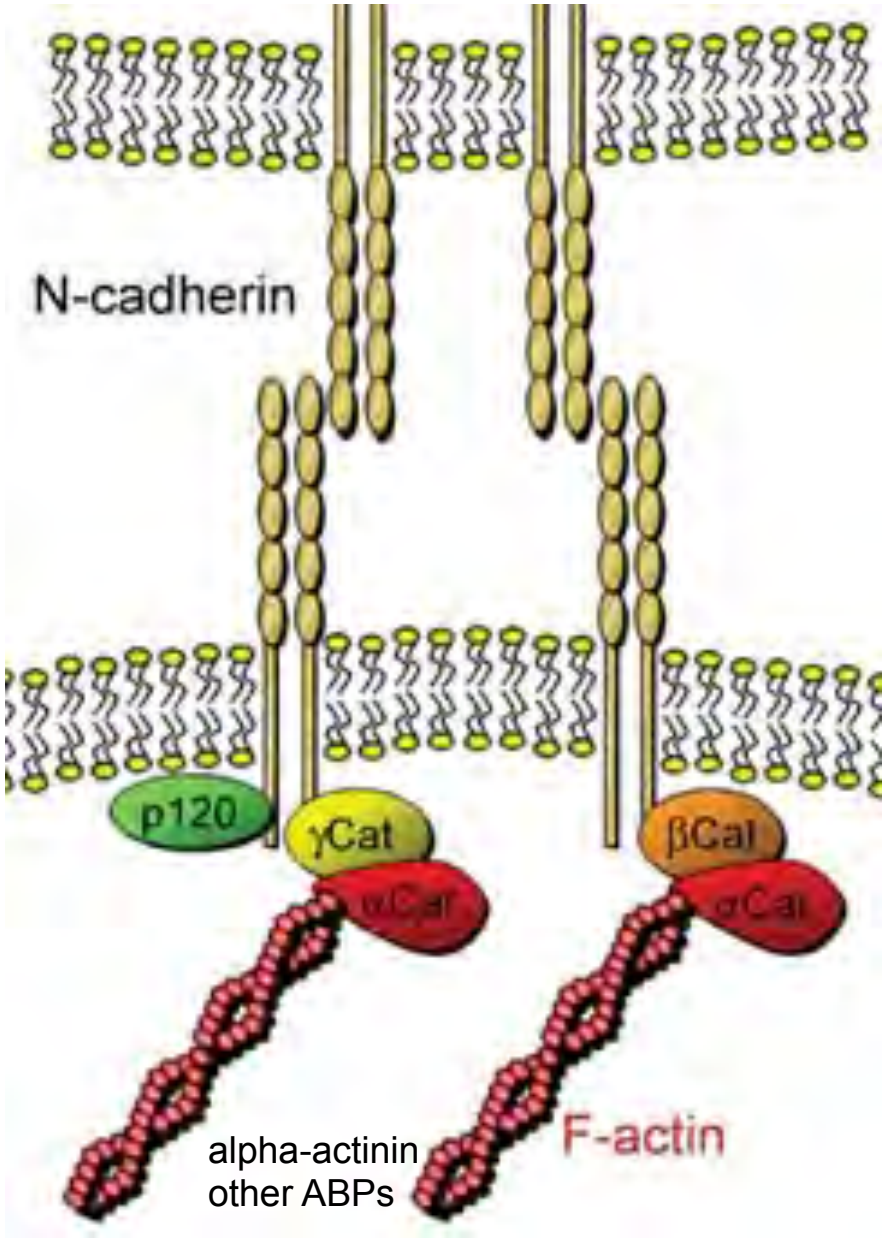
Cell movement and neurite elongation require linkage of substrate adhesions to F-actin

Several ABPs link F-actin bundles to integrin adhesions

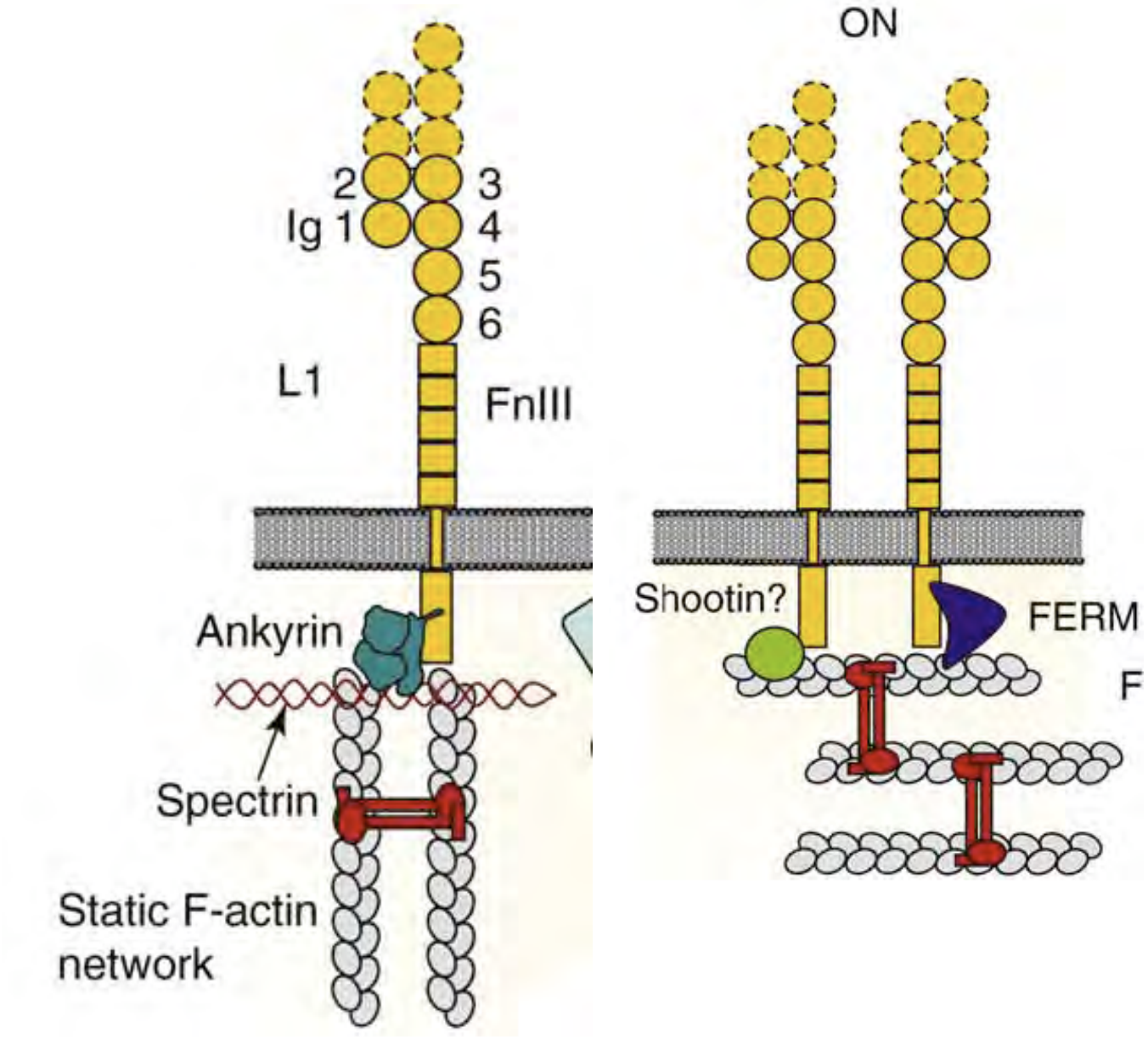
- F-actin
- alpha-actinin = F-actin linker
- vinculin = actin-integrin linker
- talin = actin-integrin linker
- Integrin dimer = adhesion receptor



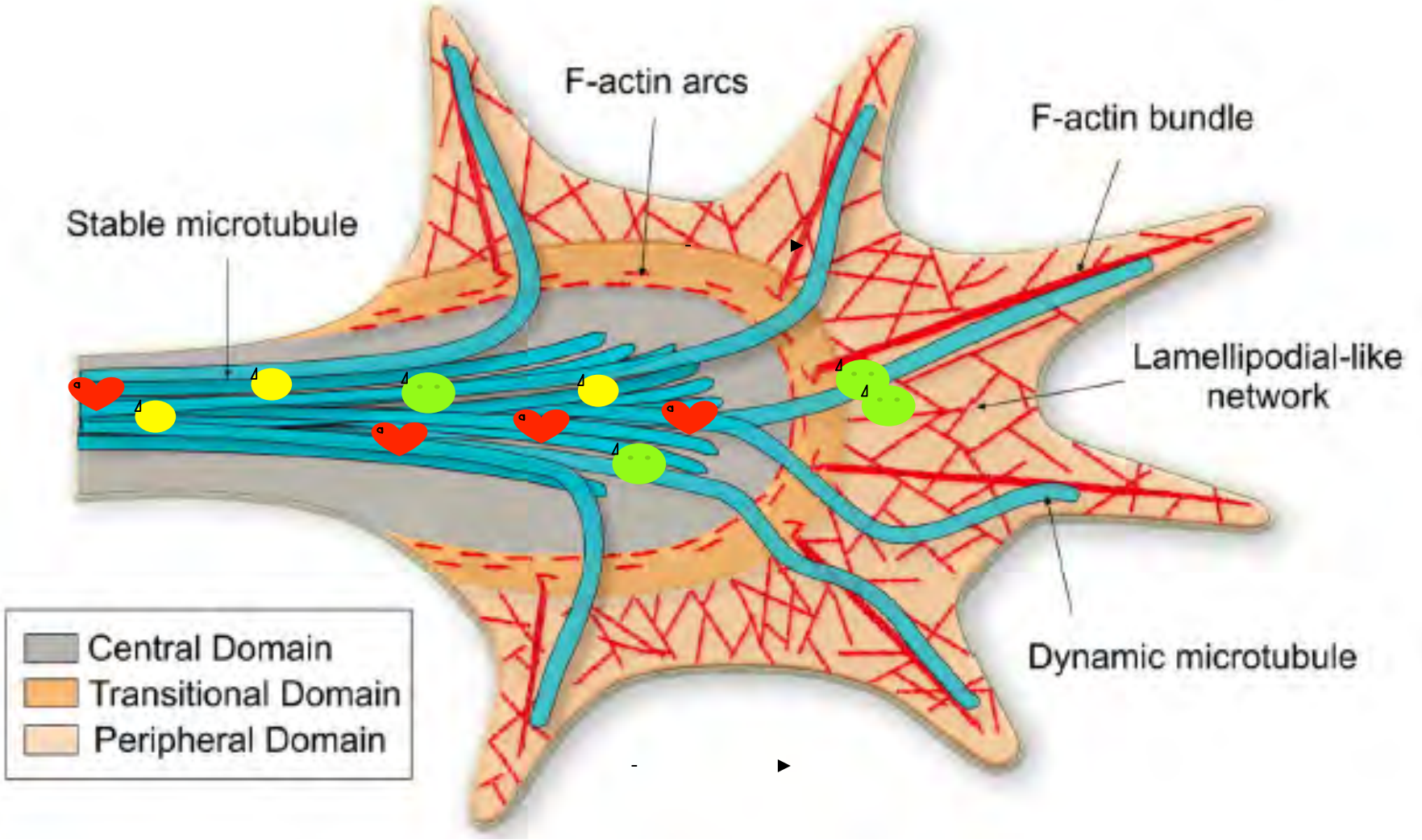
Catenins mediate linkage of N-cadherin to F-actin



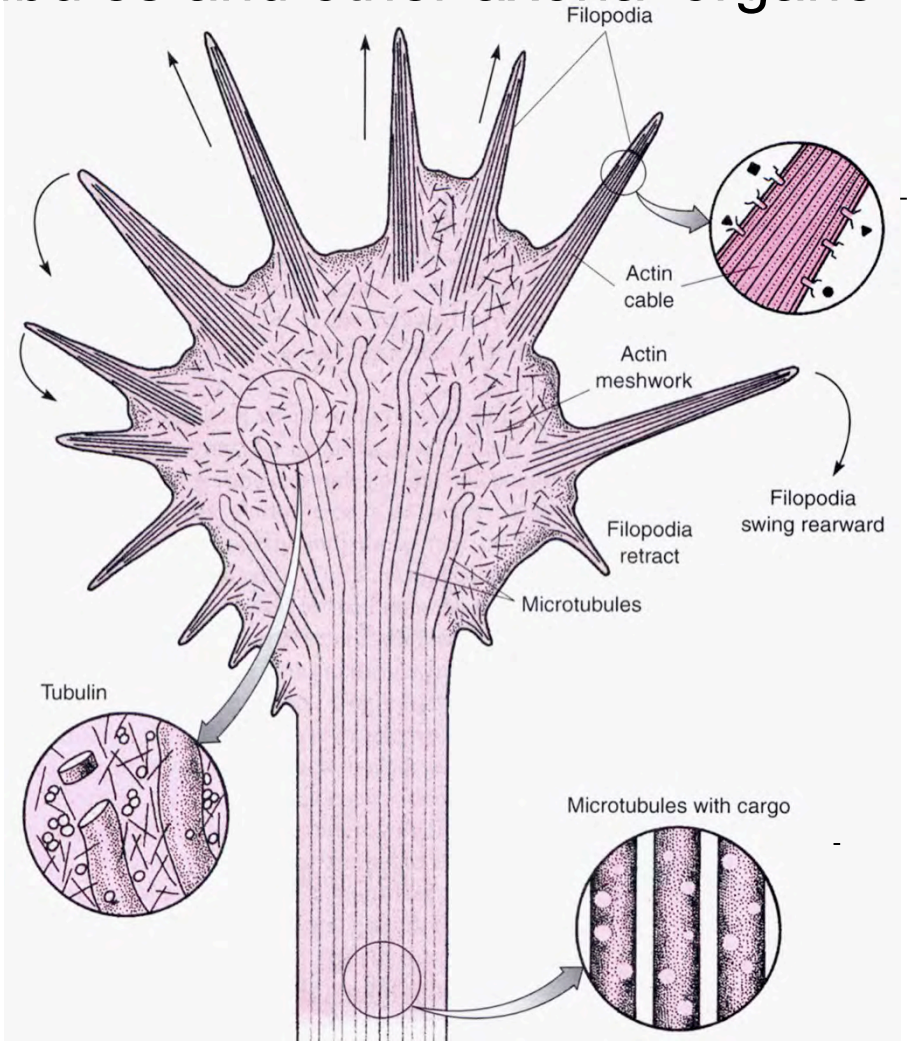
L1 and other IgCAM members link to actin via ankyrin, spectrin and FERM domain proteins.

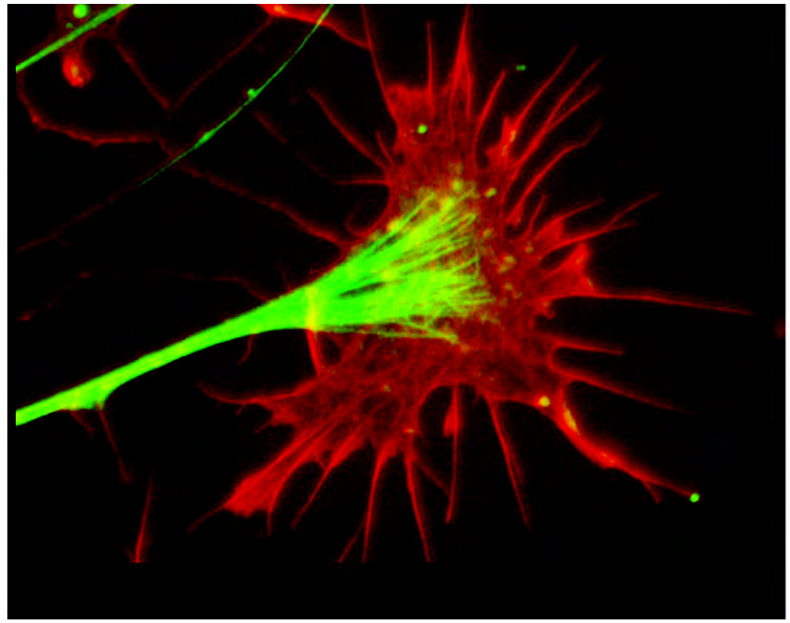


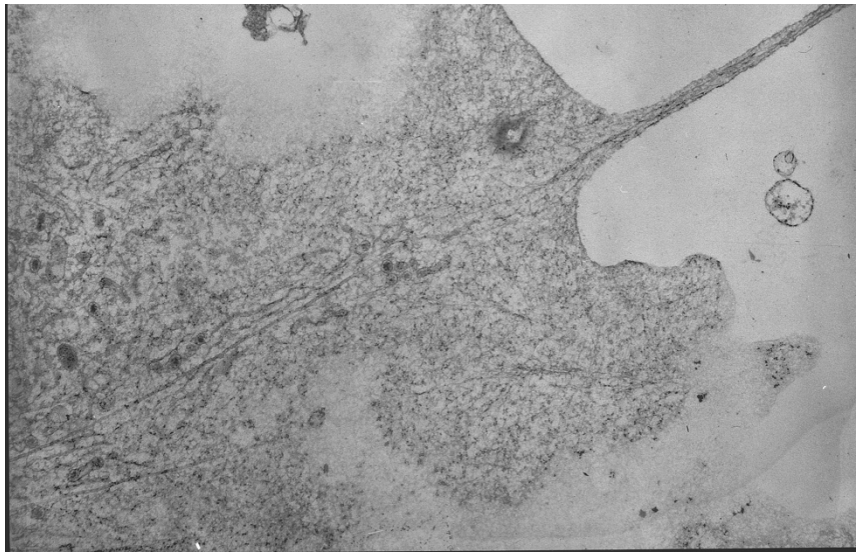
Growth cone structure is dominated by actin filaments, microtubules, and vesicular cargo transported along microtubules

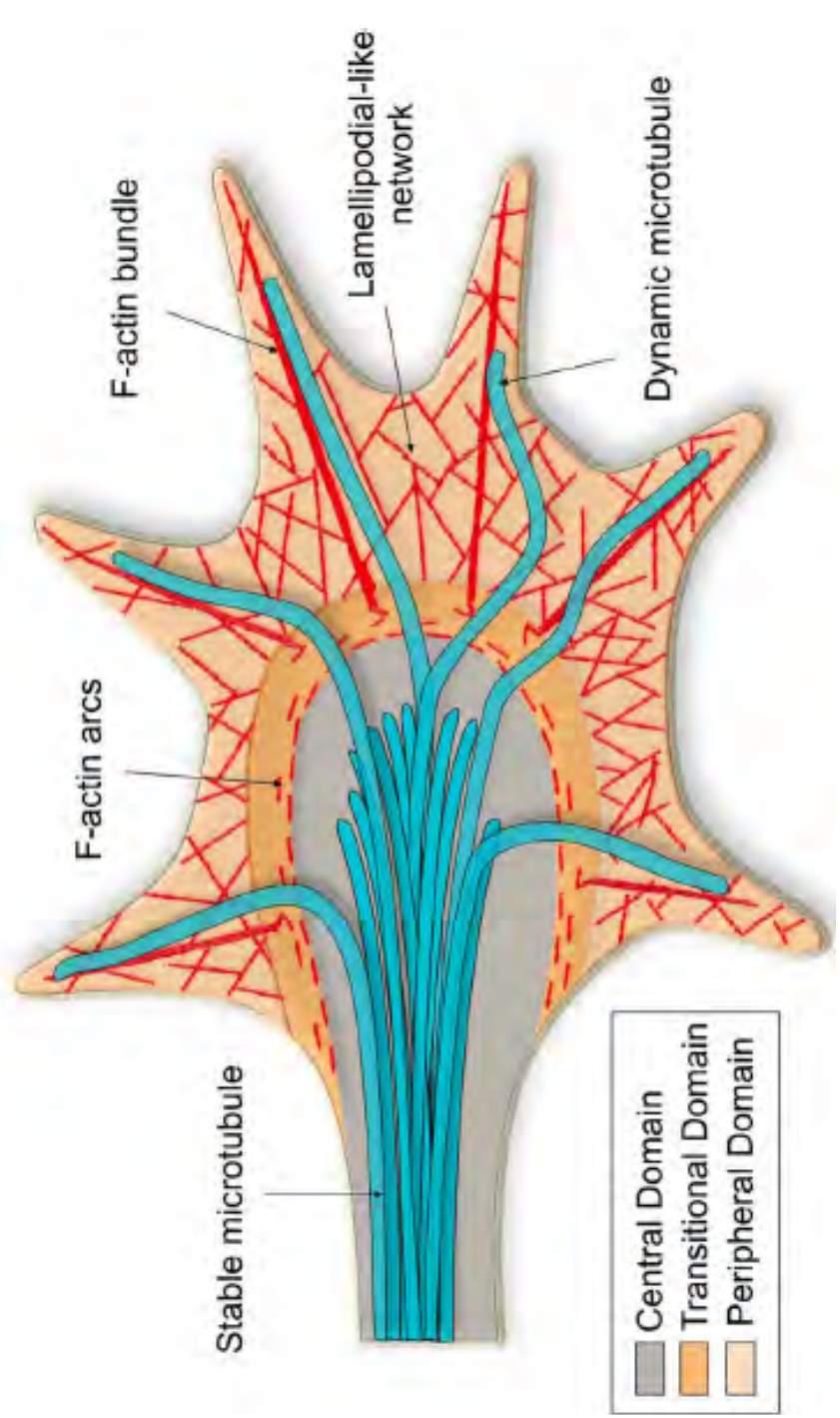


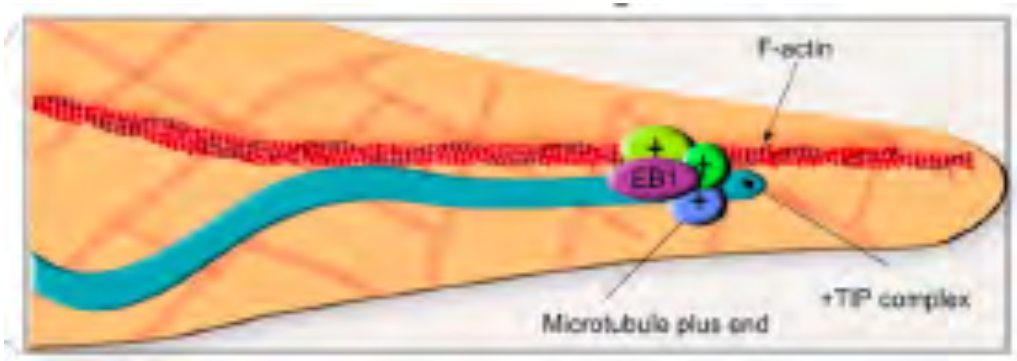
•Growth cone motility promotes neurite elongation by:
•Forming substrate adhesions that stabilize the axon.
•Protrusion of the leading margin creates space for advance of micro-tubules and other axonal organelles



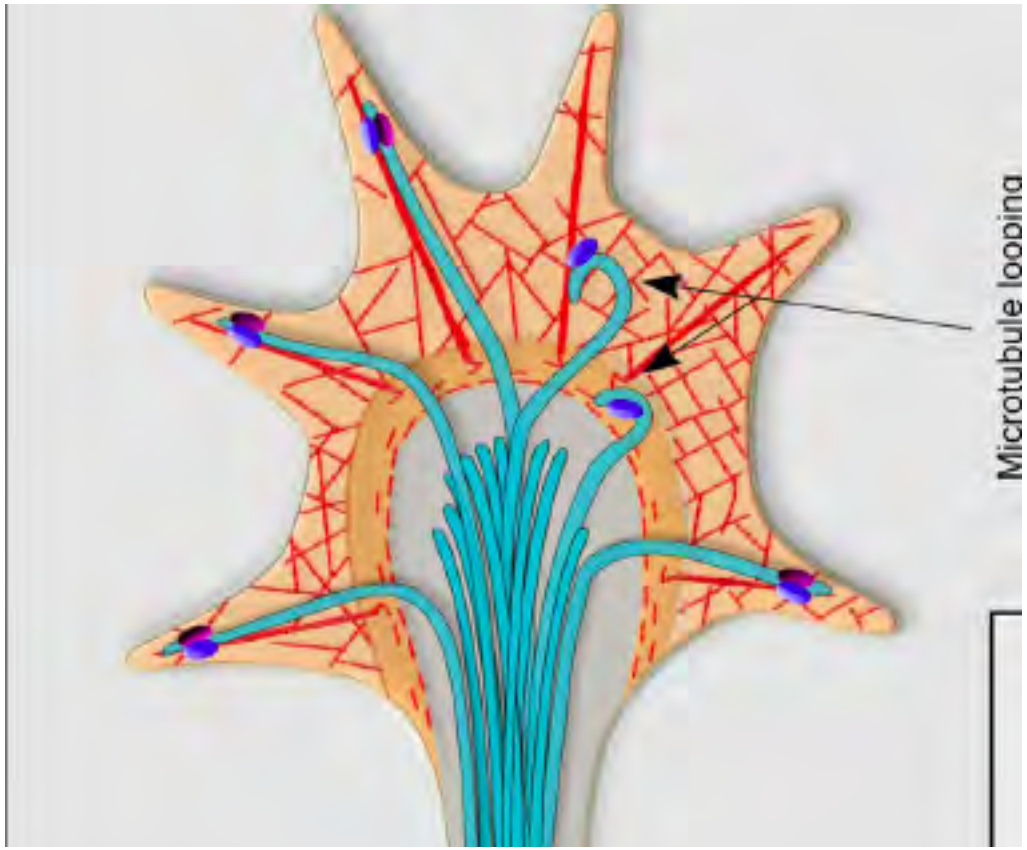


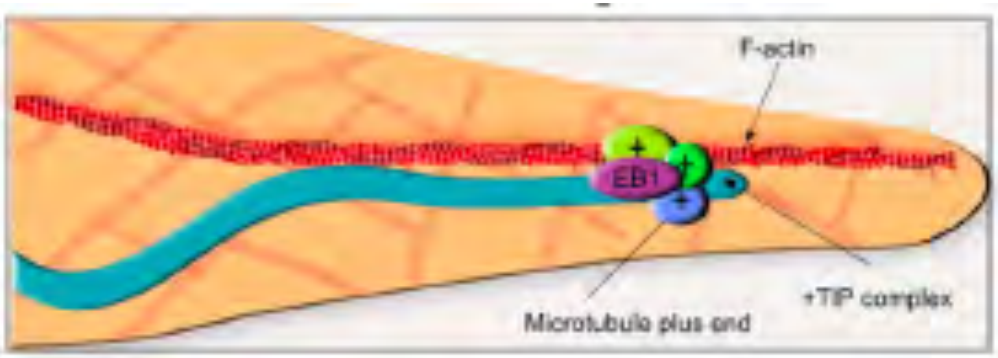






Microtubule plus-end tracking proteins (+TIPs) regulate microtubule growth dynamics and interactions with actin filaments

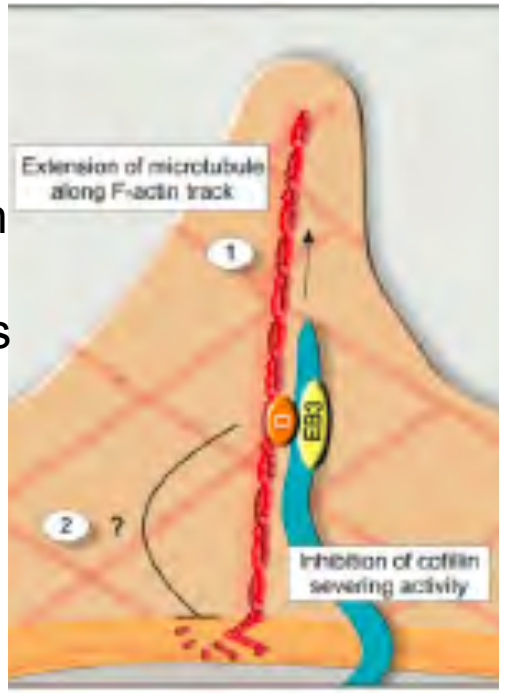




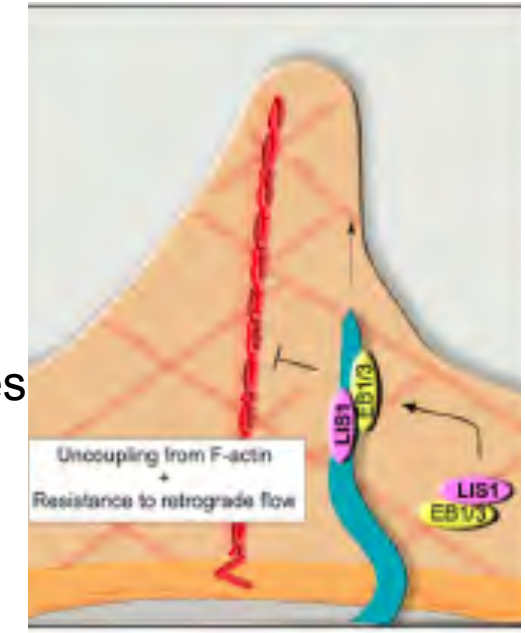
MT plus-end tracking proteins (+TIPs) regulate MT growth and interactions with actin filaments

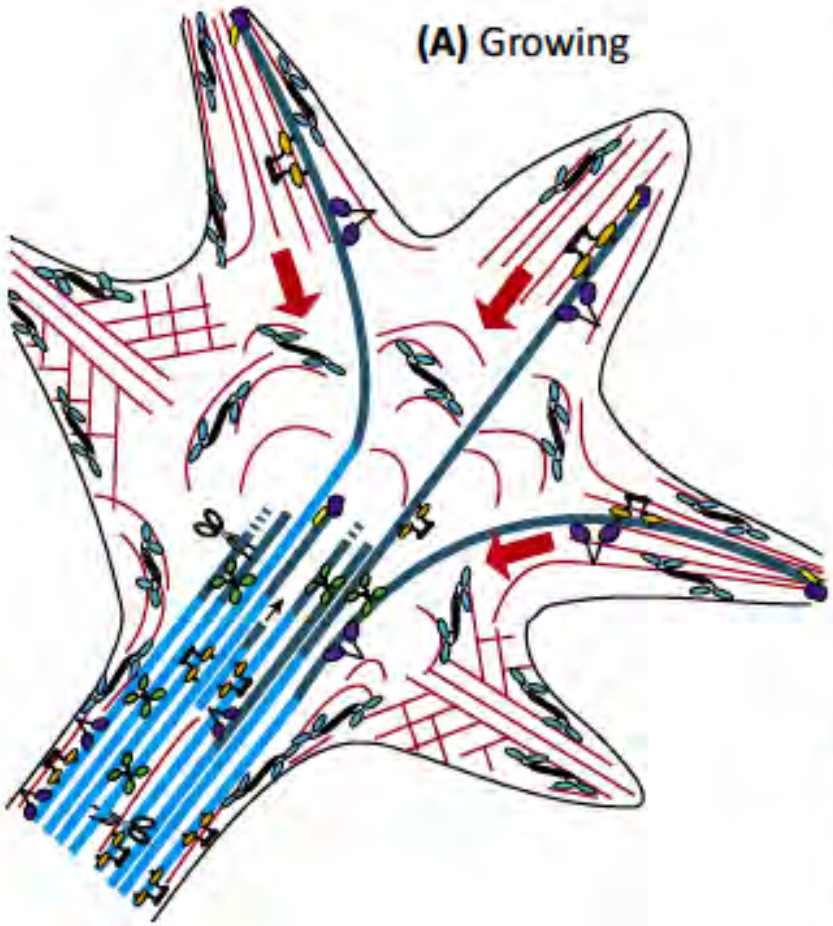
Some TIPs mediate MT retreat along retrogradely moving Actin filament bundles.

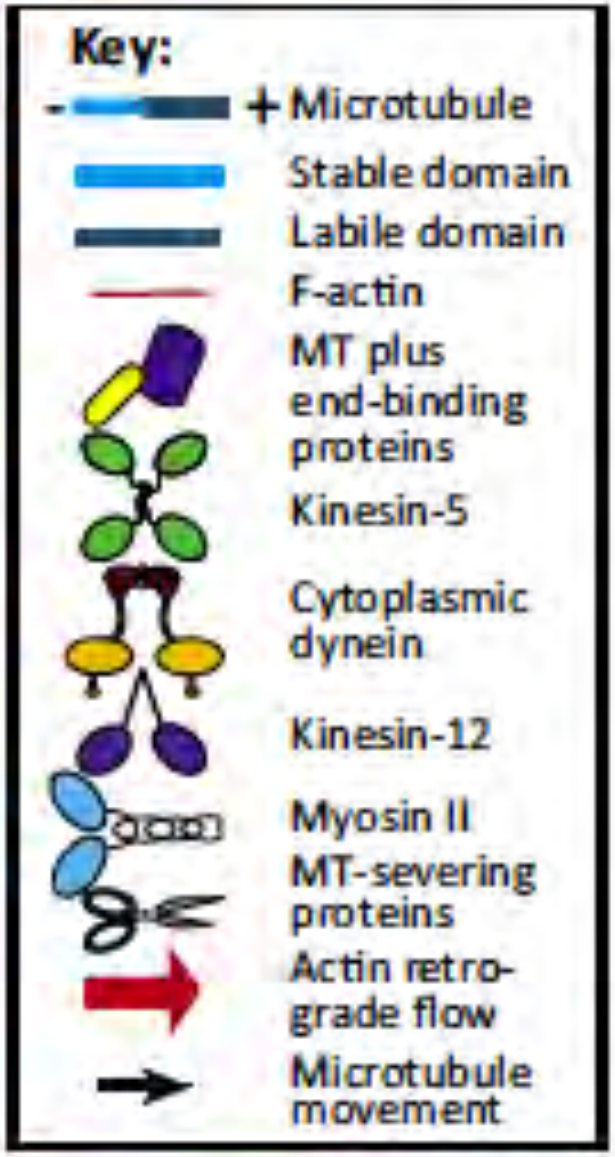
Some +TIPs mediate MT growth and/or advance along actin bundles stabilized at adhesive sites.



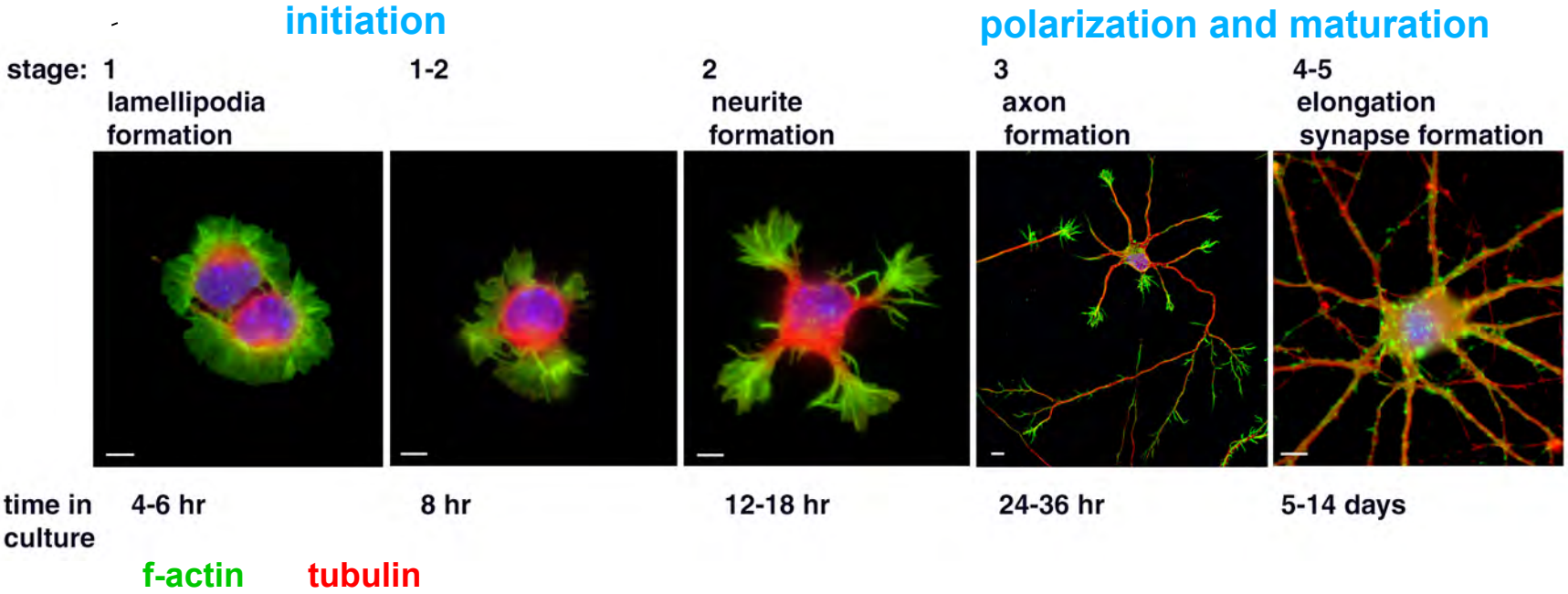
TIPs can uncouple MTs from retrogradely moving actin filaments, allowing microtubules to remain in the growth cone periphery.




(A) Growing


Key:
- + Microtubule
Stable domain
Labile domain
F-actin
MT plus end-binding proteins
Kinesin-5
Cytoplasmic dynein
Kinesin-12
Myosin II
MT-severing proteins
Actin retro-grade flow
Microtubule movement

Neuronal polarization

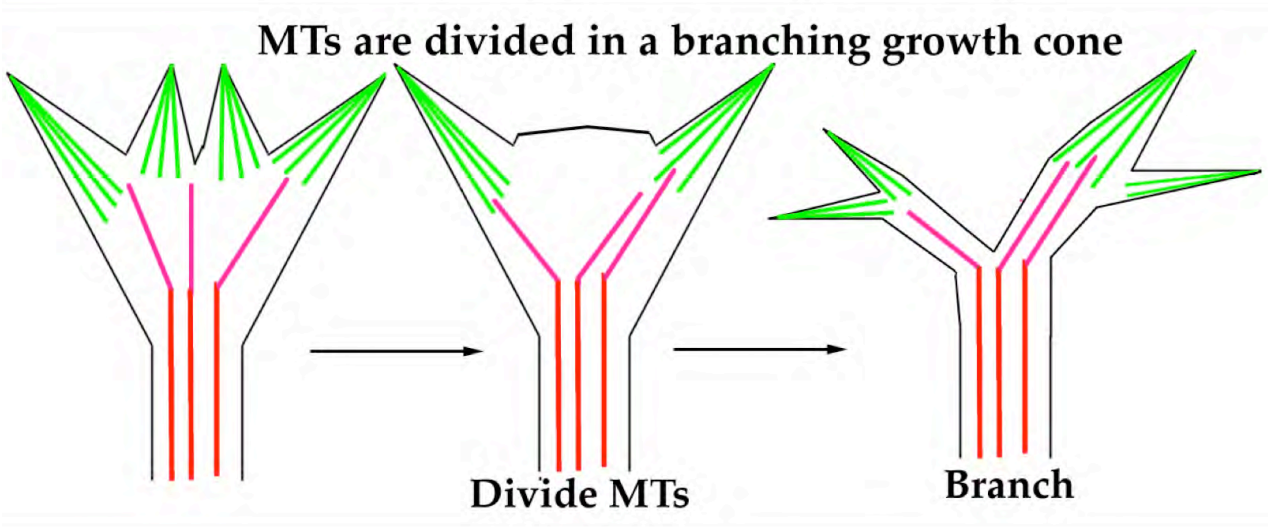


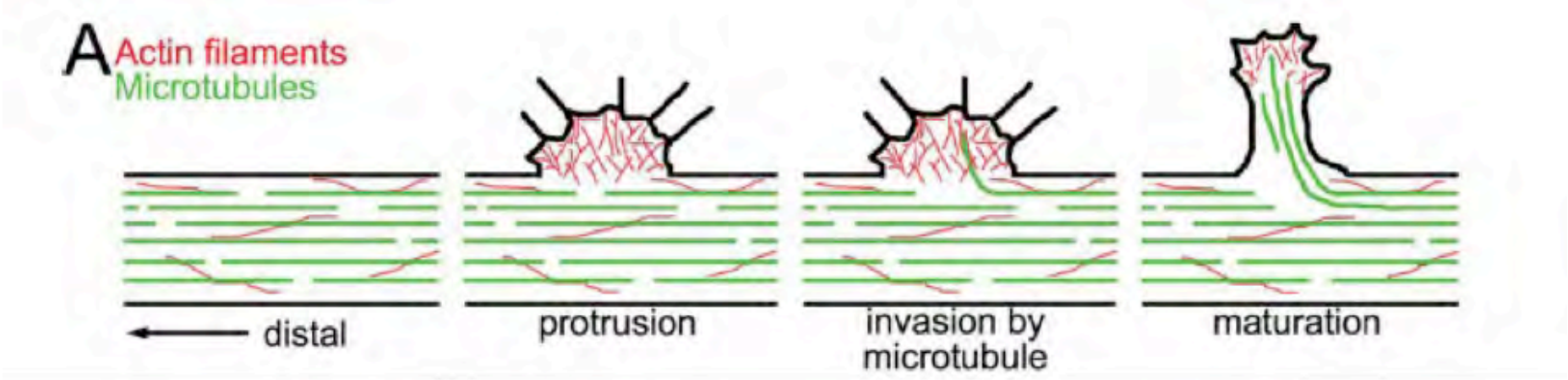
•For 40 years cultures of hippocampal neurons have been used to study neuronal polarization. Neurons first extend several neurites (stage 2).

•At stage 3 one neurite accelerates its growth rate and to become the axon.

Axons form branches in two ways:

- Division of a growth cone into multiple MT bundles
- Protrusion along axon, then MTs invade protrusion

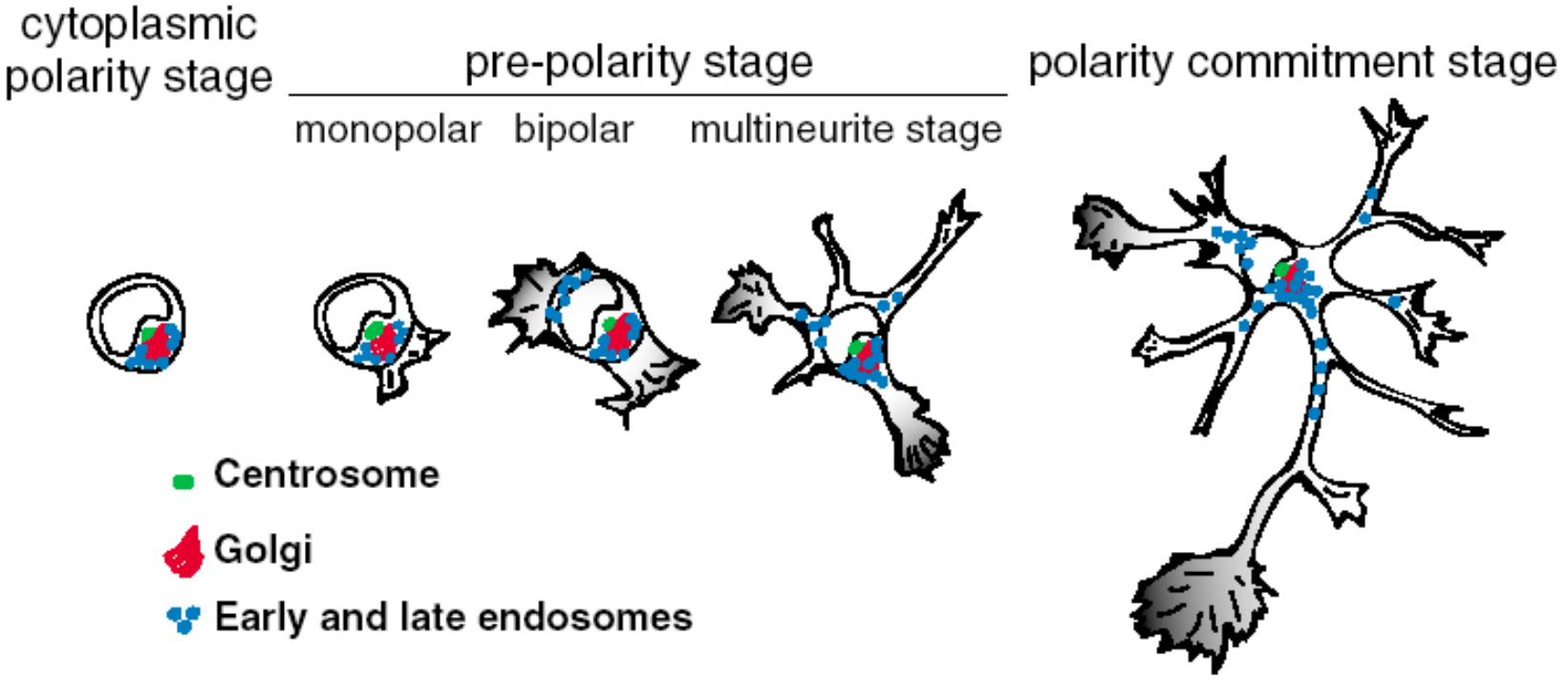




•The first-formed neurite is most likely to become the axon.

•Next most likely is the neurite at the opposite side, which is the second-formed neurite.

•This bias may be due to the orientation of the centrosome and Golgi apparatus, and their intrinsic influence on microtubule nucleation and the transport of materials into neurites.



However, extrinsic manipulations of stage 2 neurons can also induce any neurite to become the axon.

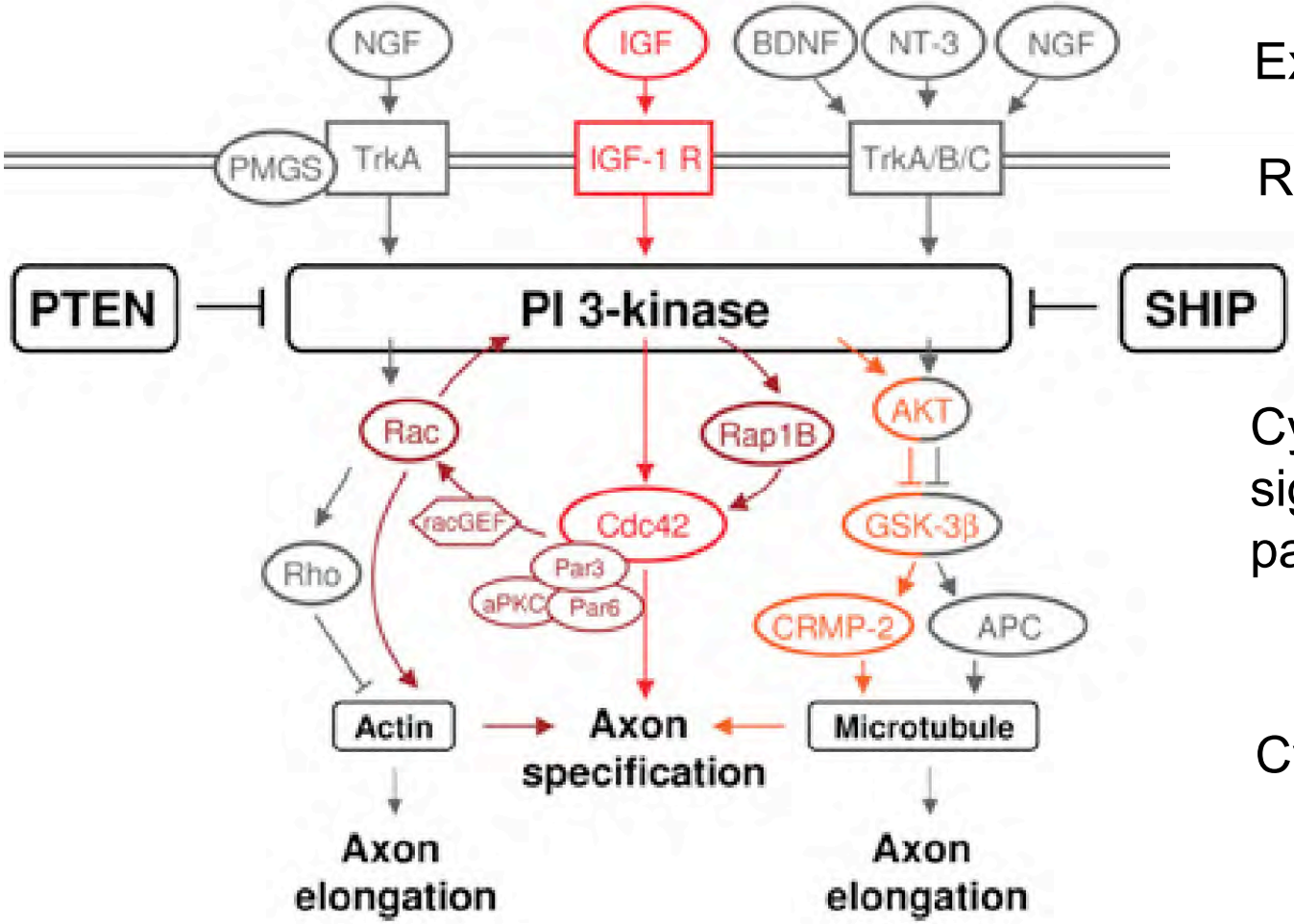
- Pull on any stage 2 neurite.
- Locally apply a microtubule-stabilizing drug to one neurite.
- Pattern the substrate with natural adhesive molecules.
- Release attractive molecules in a gradient near a neurite tip.

The environment can direct neuronal polarity by creating conditions that favor elongation of one neurite.

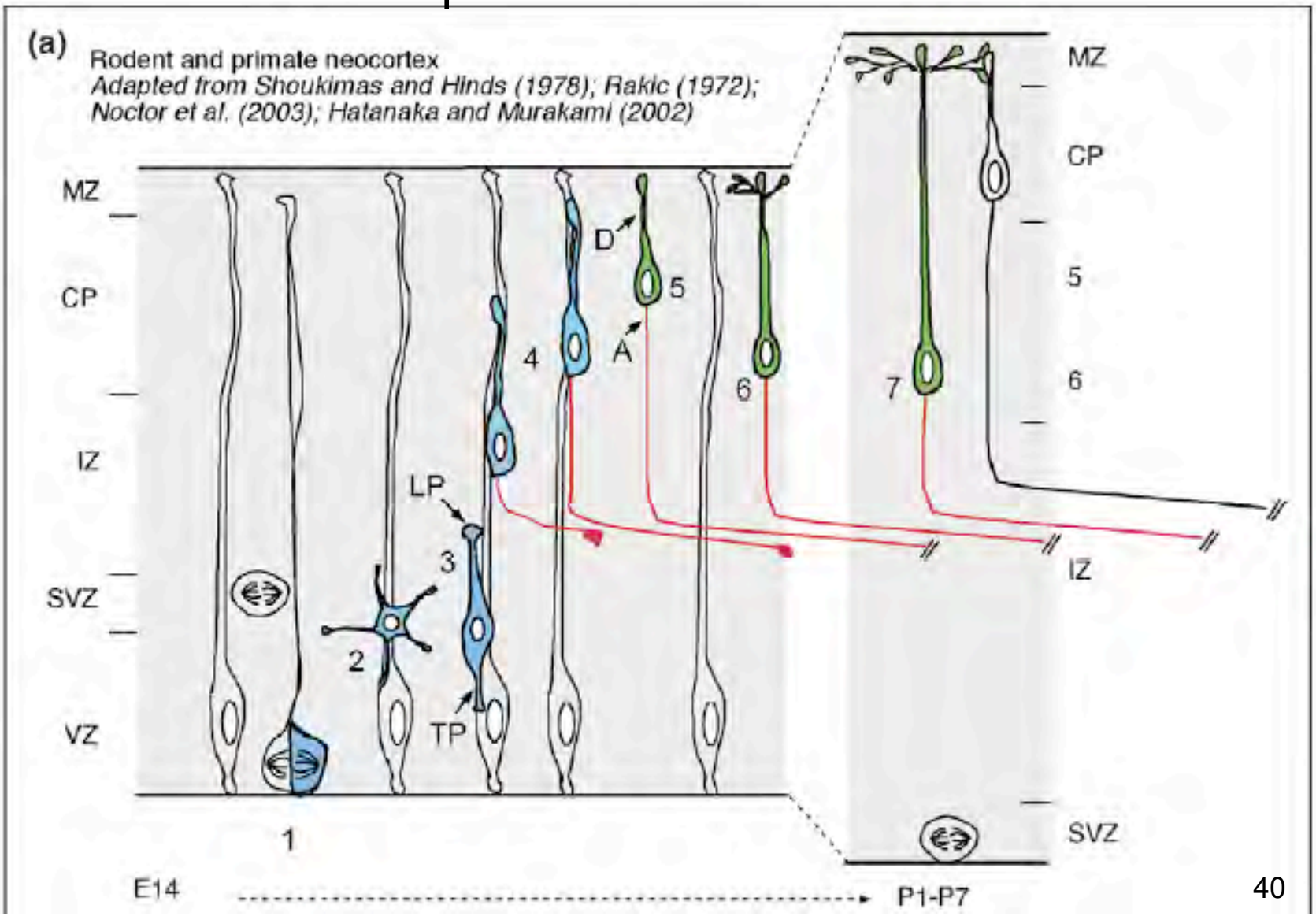
•Studies with hippocampal neurons have identified several intracellular proteins that may be involved in axonal polarization.

•These proteins are in the signaling pathways of several extrinsic cues that may regulate neuronal polarity.

•These proteins may act by regulating actin and/or microtubules.

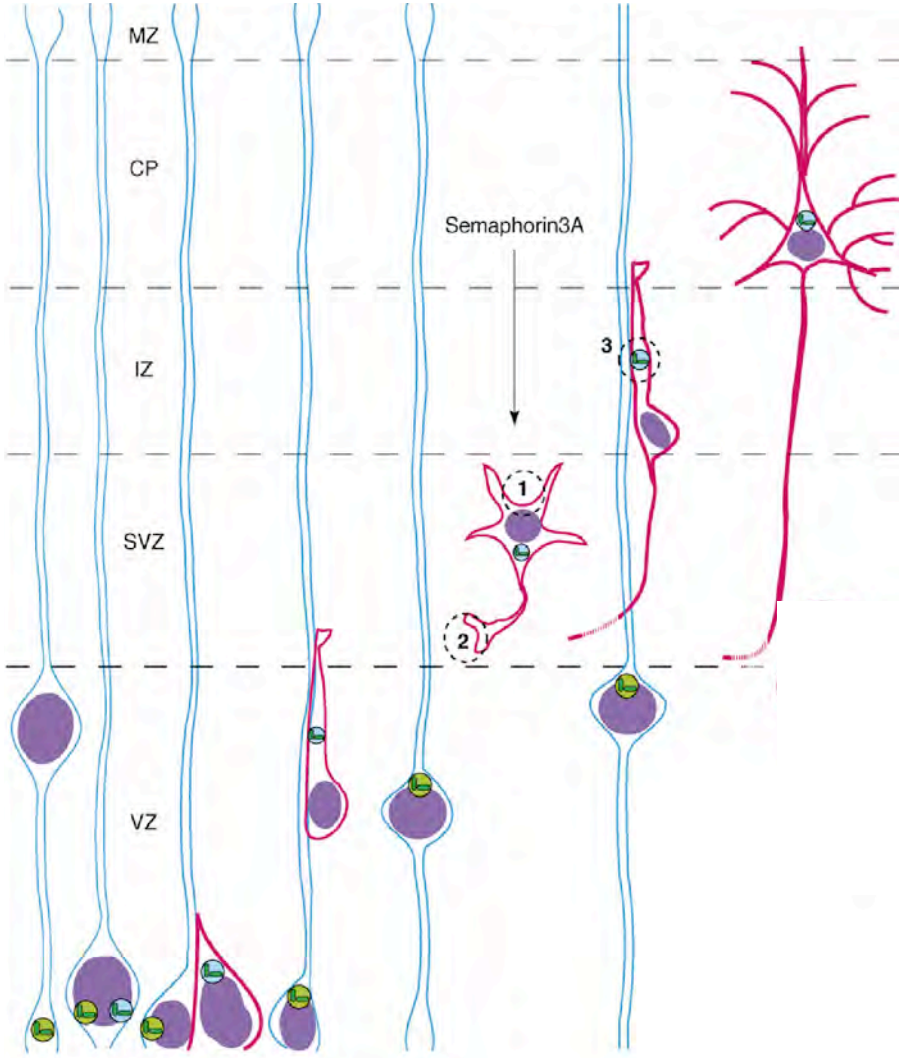


In the neocortex migrating neurons form their axon from a trailing process, while the leading process of the migrating immature neuron becomes the apical dendrite.



•Perhaps, in immature migrating neurons a combination of extrinsic cues and the intrinsic polarization of the perikaryon determine the location and orientation of the axon and apical dendrite.

•A transient relocation of the centrosome may occur in the SVZ to polarize the immature neuron and determine the axon.



Higginbotham and Gleeson (2007)

Neurite formation

- Neurite formation and elongation involve the advance of microtubules and expansion of the plasma membrane.

- A critical step in neuritogenesis is orientation and advance of microtubules into a filopodium. Forces from actin- and microtubule-motors contribute to this.

- Cell substrate adhesions are needed to stabilize neurites.

- Neuronal polarization involves the enhanced assembly and transport of components within one neurite, which becomes the axon.

- Intrinsic and extrinsic factors can influence which neurite becomes the axon.

https://www.researchgate.net/publication/312537772_Cytoskeleton_in_Axon_Growth

For a study break watch these bio- and med-based music parodies

https://www.youtube.com/watch?v=Fl4L4M8m4d0

https://www.youtube.com/watch?v=9k_oKK4Teco

https://www.youtube.com/watch?v=N5XnHtklFjI

https://www.youtube.com/watch?v=NfhSLTQTLhI

https://www.youtube.com/watch?v=eZundDVPIYw

https://www.youtube.com/watch?v=D1KXibLIOGY

https://www.youtube.com/watch?v=mHOX43-4PvE

https://www.youtube.com/watch?v=MrInh3vebSc

https://www.youtube.com/watch?v=EtAG3e3JLNI

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https://www.youtube.com/watch?v=KqaVacVpbQk