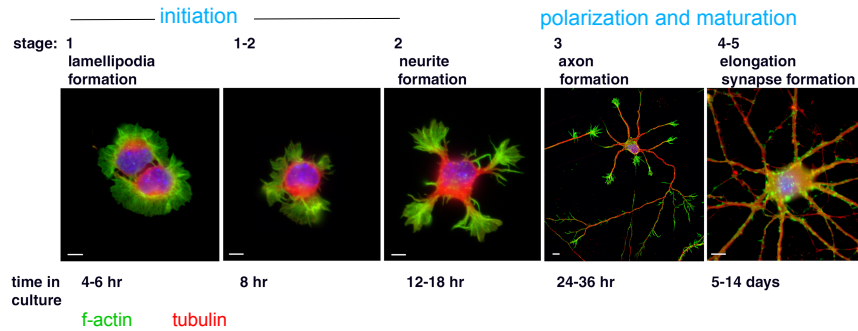


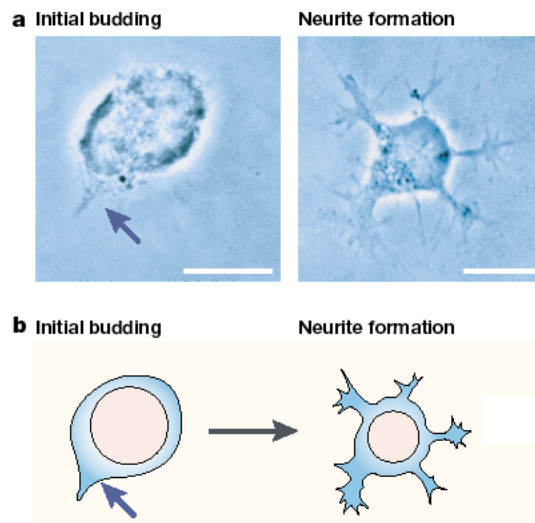
Neurite initiation

Neuronal maturation

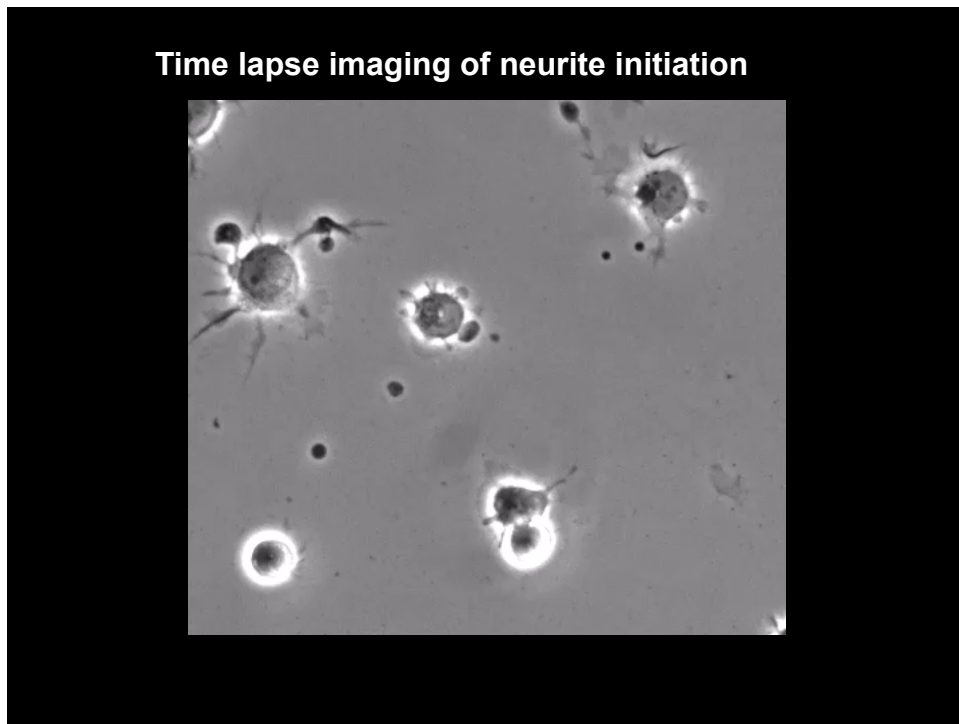


- stage 1: "spherical" neuron
- stage 2: neurons extend several neurites
- stage 3: one neurite accelerates its growth rate and matures to form the axon.
- stage 4: dendrites begin to elongate and branch
- stage 5: synaptogenesis

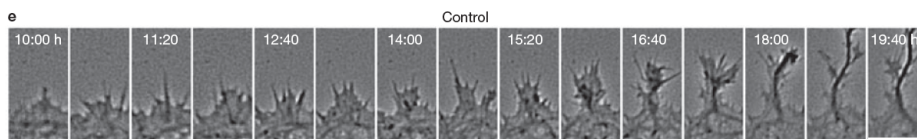
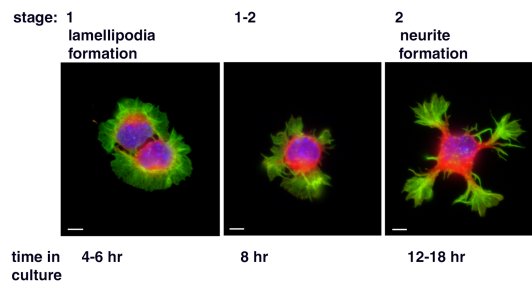
Neurite formation begins with a bud that sprouts from the cell body. One or several neurites can sprout at a time.



Neurite initiation

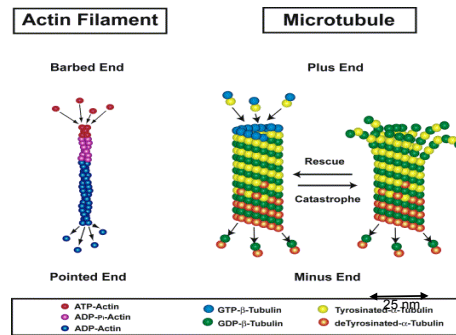


The formation of actin-based filopodia is the first step in neurite initiation



Dent et al. (2007) neuron

Neurite initiation



Actin filaments (F-actin)

- Polymers of actin monomers (globular (g) actin)
- Have intrinsic polarity: monomers add onto barbed (+) end and depolymerize from pointed (-) end.
- Polarity important in organizing networks & transport
- Polymerization requires bound ATP (actin is an ATPase)
- Actin polymerization uses ~50% of the ATP in neurons (Bernstein and Bamberg (2003))

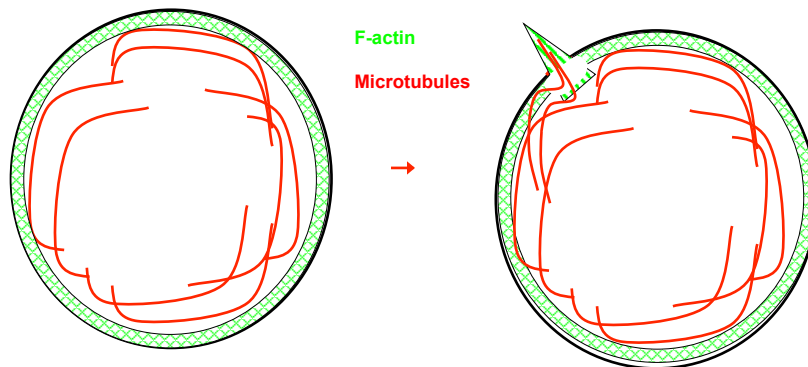
Microtubules

- polymers of tubulin dimers
- Have intrinsic polarity - important in transport
- Polymerization requires GTP bound to tubulin dimers
- Plus ends alternate between states of growing and resting.
- Minus ends depolymerize
- catastrophe = "peeling apart" of filament strands

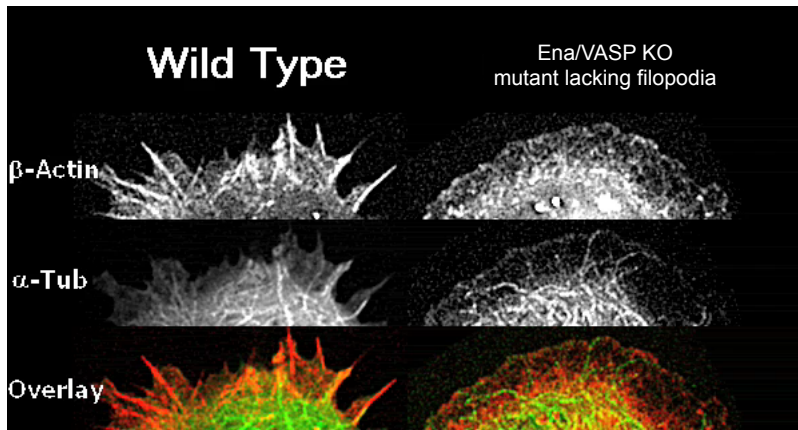
A critical step in neurite initiation is the establishment of an axis of microtubule orientation and transport

Symmetry of perikaryon

The symmetry is broken!
Microtubules enter the neurite bud.



Microtubules “break the sphere” and drive neurite initiation

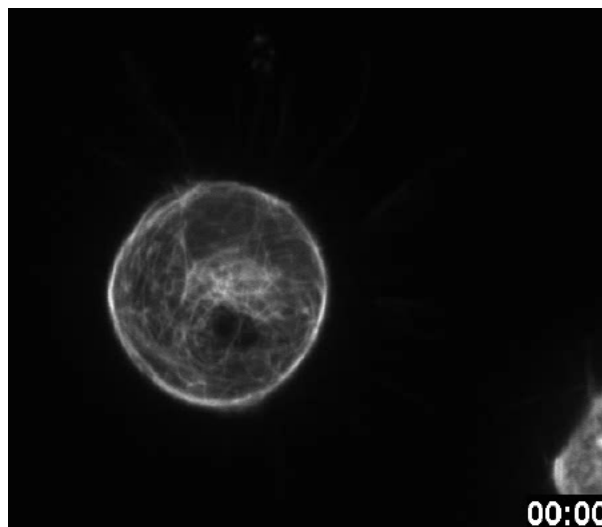


F-actin
microtubules

mmvvee=Mena/VASP/EVL triple ko

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

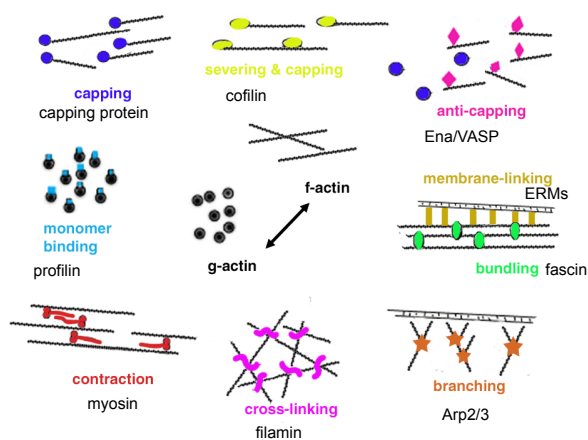
Over expression of EGFP-Map2c induces neurite formation in Neuro2A cells



Functions of actin filaments in neurons

- Provide structural integrity to filopodia and dendritic spines
- Regulate organization of membrane proteins and associated protein complexes
- Polymerization drives protrusion of filopodia and lamellipodia
- Substrate for intracellular transport
- Involved in targeting of neuronal components
- Transmission of mechanical force
- Involved in synaptic endocytosis & exocytosis

Actin-binding proteins

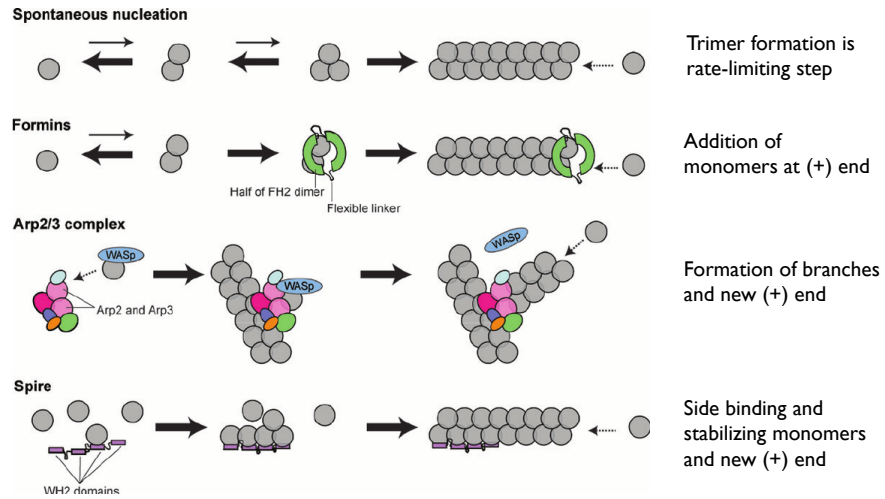


• Regulate actin polymerization and organization

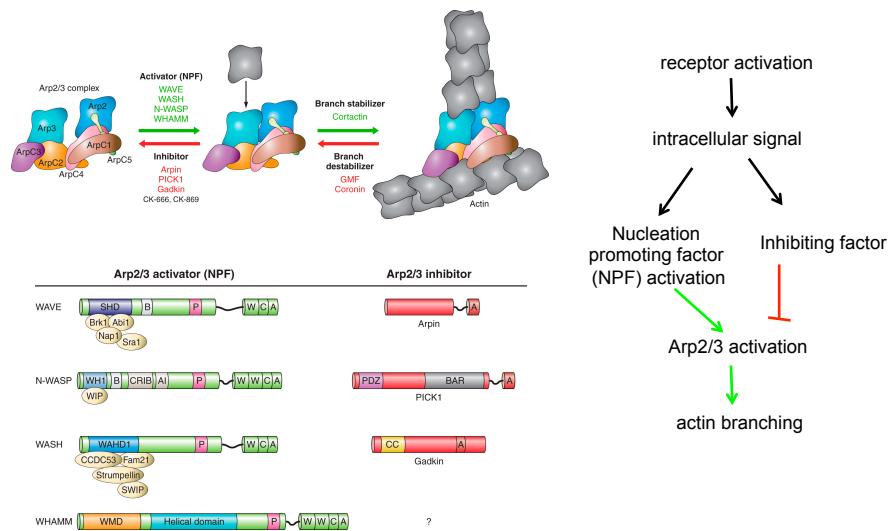
• Functional outcome depends on

- localization
- relative concentration of other regulatory proteins

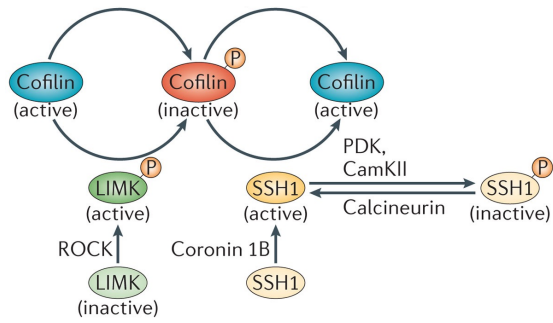
Mechanisms of actin nucleation



Regulation of Arp2/3: Activation

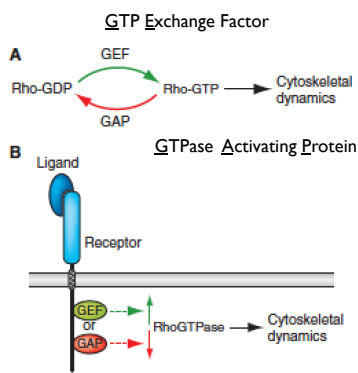


Regulation of cofilin activity

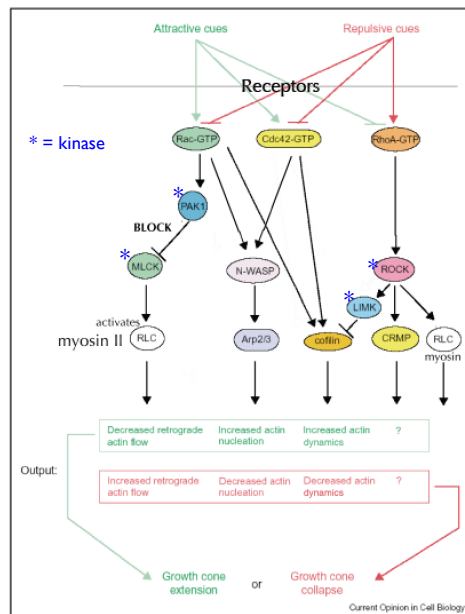


Nature Reviews | Molecular Cell Biology

Rho, Rac & cdc42: collapse vs. extension



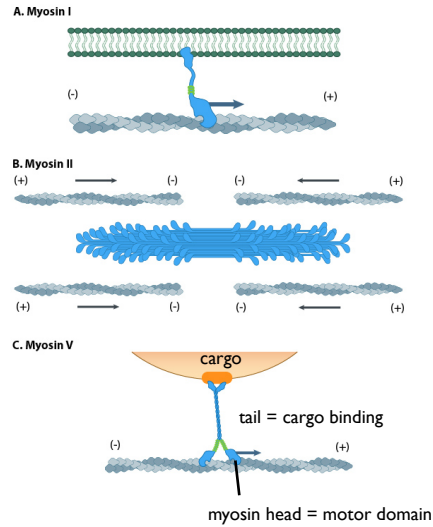
- an activation/inhibition step may be 1:1 or can amplify the signal



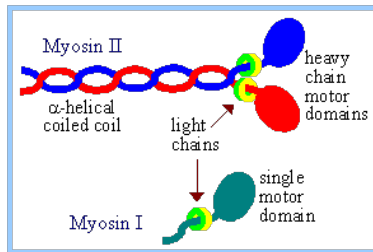
Actin based motors: myosins

Each myosin molecule:

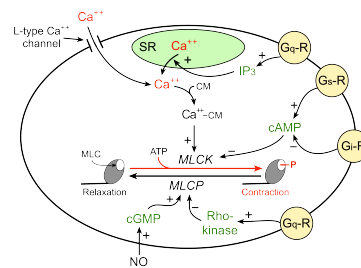
- binds/moves on a single filament
- moves in only one direction on the filament (most myosins move toward the (+) end, myosin VI moves toward (-) end)
- BUT, the outcome of the motor activity depends on
 - motor and cargo anchoring
 - cytoskeletal orientation



Regulation of myosin activity

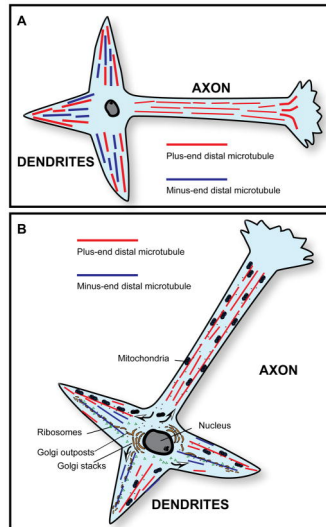


- Myosin light chain (MLC) regulates myosin movement (in non-muscle myosins)
- Phosphorylation of MLC at ser19 leads to increased myosin activity (i.e. movement along actin filament)



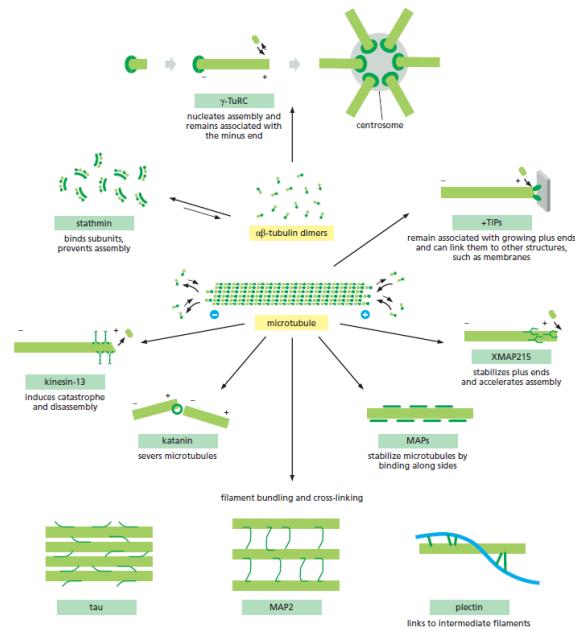
- MLCK kinase (MLCK) is activated by binding to the Ca²⁺-calmodulin complex.
- Phosphorylation of MLC Kinase by Pak (downstream of Rac/cdc42) reduces PLCK activity, thus reducing MLC activity
- Phosphorylation of MLC Phosphatase by Rho Kinase reduces PLCP activity, thus increasing MLC activity & contraction

Microtubules in axons and dendrites



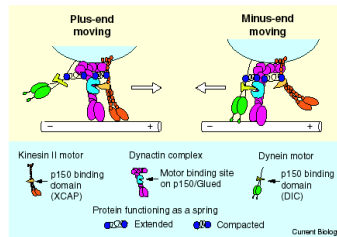
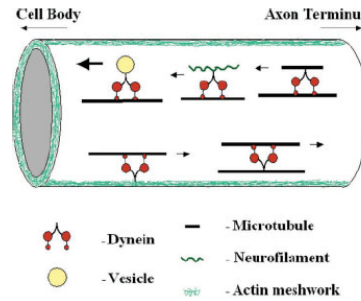
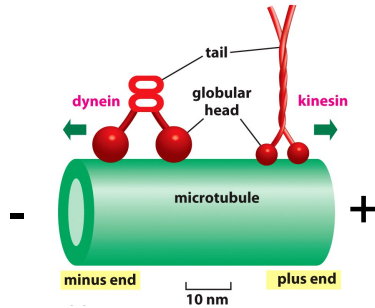
- Axons have MTs with (+) end distal
- Dendrites have mixed polarity MTs
- Required for movement of nucleus during cell migration
- Provide structural rigidity to axons and dendrites
- Major substrate for long distance transport
- Important for targeting of neuronal organelles metabolites

Microtubule Associated Proteins (MAPs)



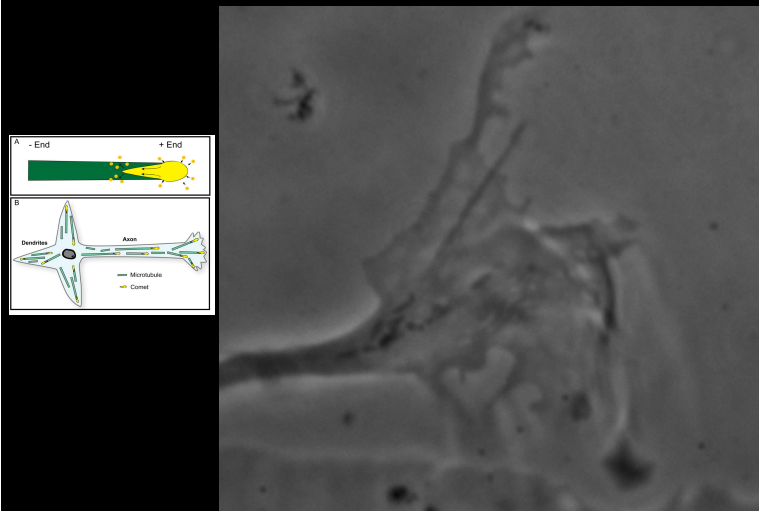
<http://reasonandscience.heavenforum.org/t2096-the-astonishing-language-written-on-microtubules-amazing-evidence-of-design>

Microtubule based motors



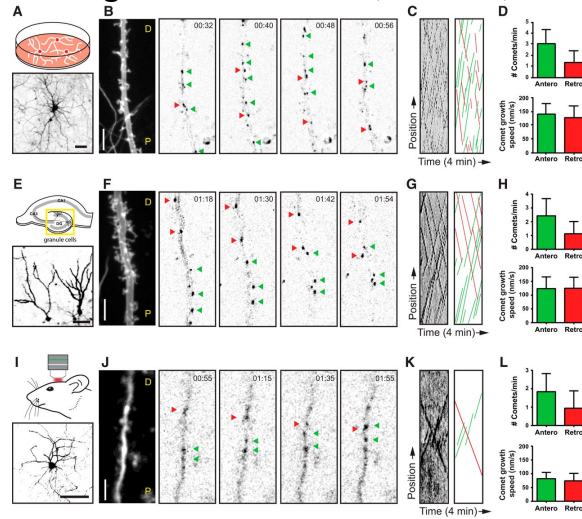
- Can move either the cargo or the MT
 - depends on which is more "anchored"

Microtubule dynamics can be visualized using EB3-EGFP "comets"



Sarah Wanner

Using GFP-EB1 to characterize mixed dendritic microtubule organization in vitro, ex vivo, and in vivo.



Kah Wai Yau et al. *J. Neurosci.* 2016;36:1071-1085

J. Neurosci. The Journal of Neuroscience
©2016 by Society for Neuroscience

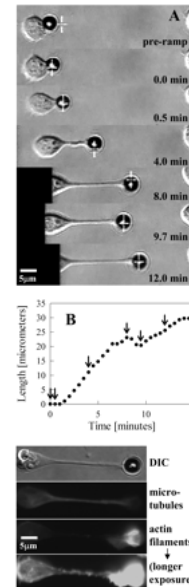
Neurite formation requires tension

Local application of tension to the (external) edge of a cell soma can induce neurite formation, suggesting that local (internal) tensions generated in the actin cortex may promote neurite formation.

source of tension:

internal- push \leftrightarrow external - pull

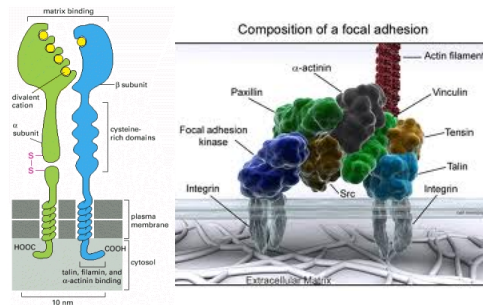
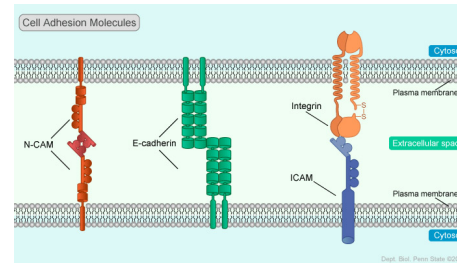
- requires coupling (adhesion)
- cytoskeleton-membrane
- membrane-substrate



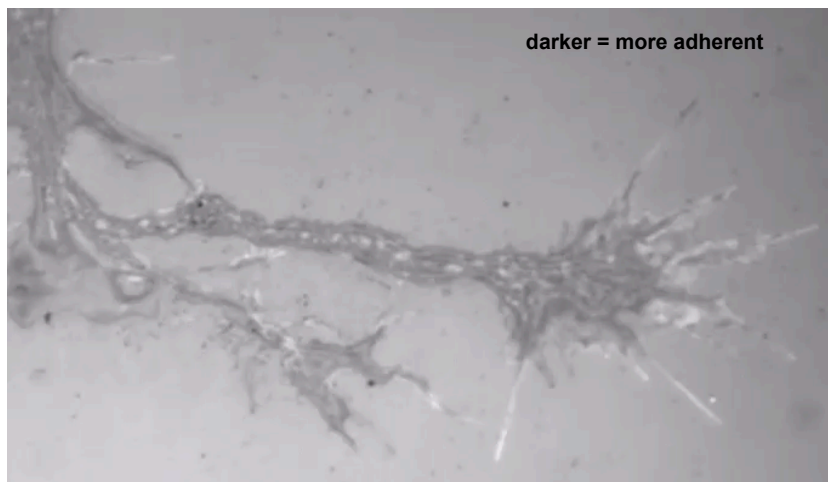
Fass & Odde (2003) *Biophys J* 85(1):623-36

Cell Adhesion Molecules (CAMs)

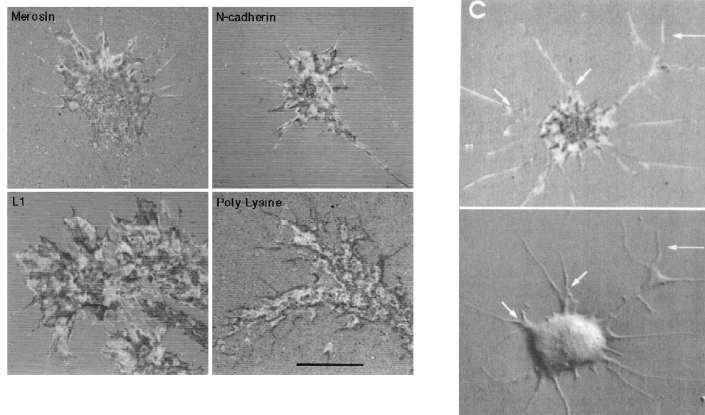
- can be involved in
 - cell-cell adhesion
 - cell-substrate adhesion
- are physically linked to the cytoskeleton (usually to f-actin)
- "engagement" of a CAM triggers intracellular signaling events
- CAM di/oligomerization will affect ligand binding and intracellular linkage



Adhesion dynamic can be visualized using Interference Reflection Microscopy (IRM)

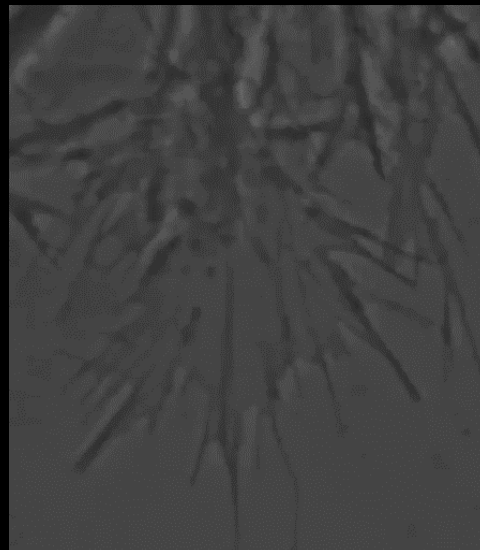


Adhesion patterns depend on substrate



Cellular adhesions to in vitro surfaces are seen with interference reflection microscopy, appearing as discrete dark regions beneath the cell membrane.

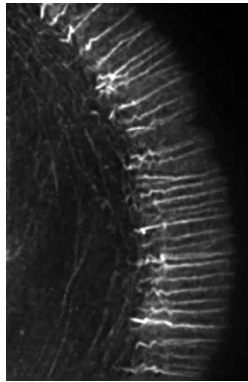
Membrane protrusion is coupled to retrograde flow



Neurite initiation

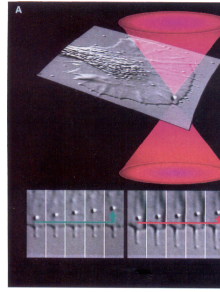
Retrograde flow

During membrane protrusion, filamentous actin at the leading edge of the cell undergoes continuous retrograde flow



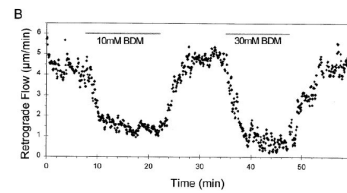
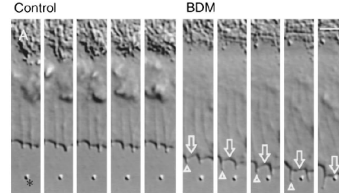
movie of fluorescently labeled actin

Retrograde flow stops, and filopodia protrusion is enhanced, when the force is applied via adhesion to the cell surface



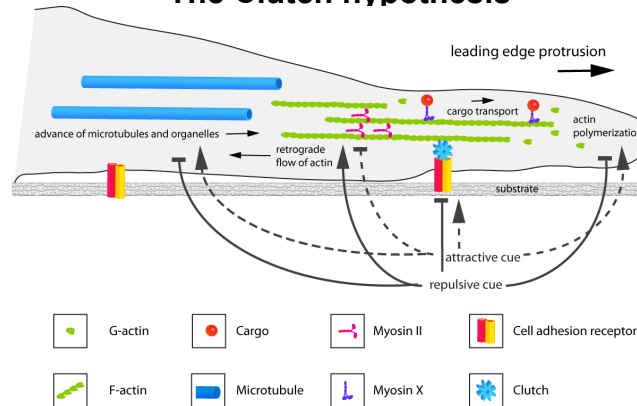
A "sticky" bead places on the cell surface moves retrograde (left). If the bead is retrained with a laser trap (right), filopodia anterograde to the bead are able to protrude/extend.

Actin retrograde flow requires myosin II activity and is blocked by application of the myosin inhibitor BDM



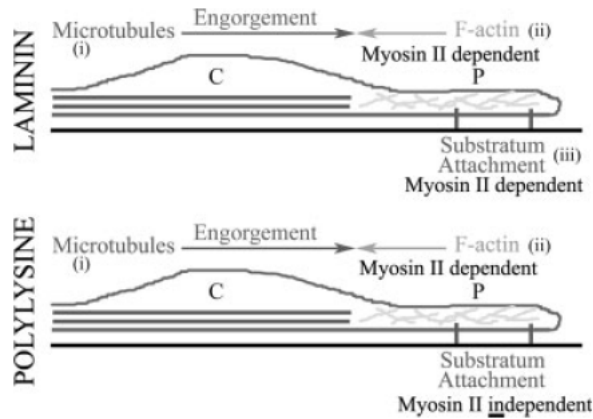
Meideros et al (2006) Nat. Cell Biol
 Lin et al (1997) Biol Bull
 Lin et al (1996) Neuron

The Clutch hypothesis



- Filopodial protrusion is driven by actin assembly at the filopodial tip
- A clutch mechanism holds the F-actin linked to the substratum.
- When the clutch is not engaged, retrograde flow, powered by myosin II, pulls F-actin back.
- When the clutch is engaged, actin polymerization leads to protrusion and movement by other motors brings material (e.g., microtubules, membrane, actin monomers) toward the leading edge.
- Different myosin motors are involved in these movements in different directions.

The roles of myosin II in substrate adhesion and retrograde flow are distinct and substrate dependent

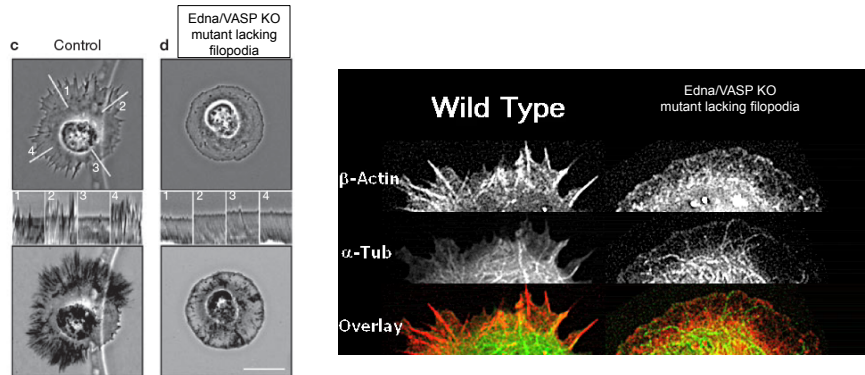


Effect of myosin II inhibition

- retrograde flow reduced
 - adhesion reduced
 - actin polymerization not sufficient to drive filopodia protrusion, may even get retraction
-
- retrograde flow reduced
 - no change in adhesion
 - actin polymerization sufficient to drive filopodia protrusion, growth cone advances

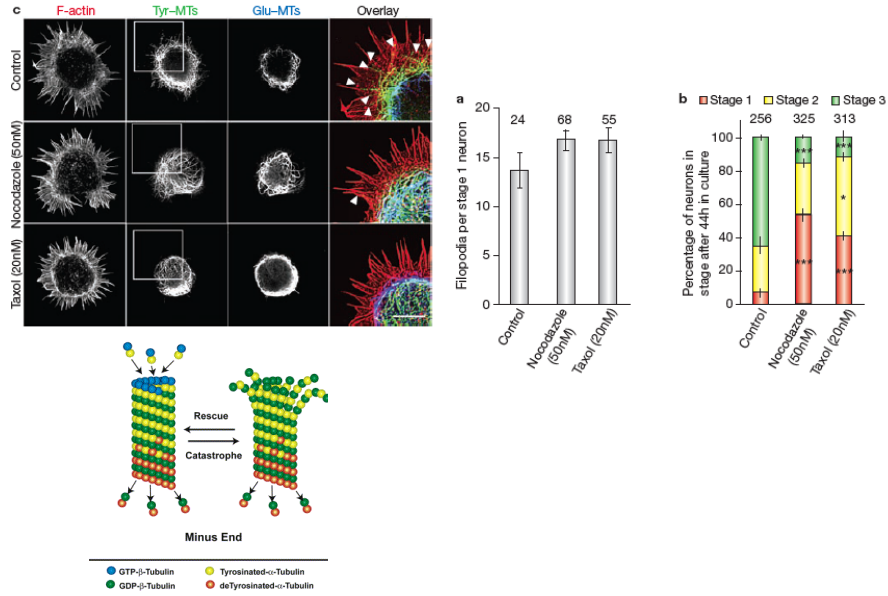
Ketschek et al. (2007) Developmental Neurobiology 67(10):1395

Ena/VASP protein anti-capping is required for filopodia formation



Dent et al. (2007) neuron

Neurite initiation requires dynamic microtubules



Neurite initiation: coupling protrusion and adhesion

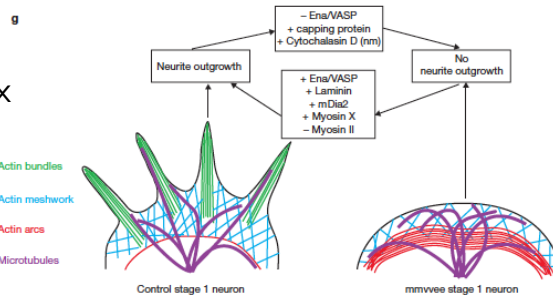
Defects in Ena/VASP triple KO:

Rescued by:

- plating on laminin & engaging integrins
- overexpressing mDia or myosin X (F-actin barbed end binding)
- inhibiting myosin II (reduced retrograde flow)

Mimicked by:

- actin capping drug
- overexpressing capping protein

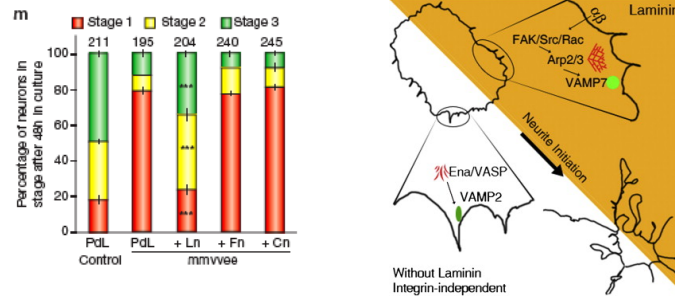


• Ena/VASP actin-binding proteins (anti-capping) proteins are critical for filopodia initiation, but...

• The requirement for Ena/VASP proteins in filopodia formation is substrate dependent (e.g. CNS vs. PNS)

• Requirement for Ena/VASP proteins can be rescued by treatments that enhance filopodia formation OR decrease retrograde flow

Neurite initiation: coupling protrusion, adhesion and exocytosis



- Different substrates may activate different intracellular signaling cascades and activate different actin-binding proteins that can drive filopodia formation
- VAMP2 & VAMP7 are vSNAREs that are involved in different types of exocytosis
- ena/VASP dependent neurite initiation is integrin-independent and requires VAMP2
- in the absence of ena/VASP, Arp2/3 can drive neurite initiation, but this requires integrin activity (& substrate) and VAMP7
- exocytosis is critical, but what "substrate" is exocytosed not known.....
 - secreted, membrane associated and transmembrane
- This is not the first time we've seen substrate dependence

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

Gupton & Gertler, Dev Cell 18, 725–736 (2010)

Neurite Initiation

- Filopodia formation is the first step
- Actin dynamics must be coupled to
 - dynamic microtubules
 - substrate adhesion
 - a "clutch"
 - exocytosis
 - retrograde flow
- The actual proteins "required" for neurite initiation is context dependent
 - e.g. effects of Ena/VASP depletion would not be apparent if the experiment is done in cortical neurons plated on laminin (a very common method) or in a neuron type that expressed mDia.