Axon guidance II

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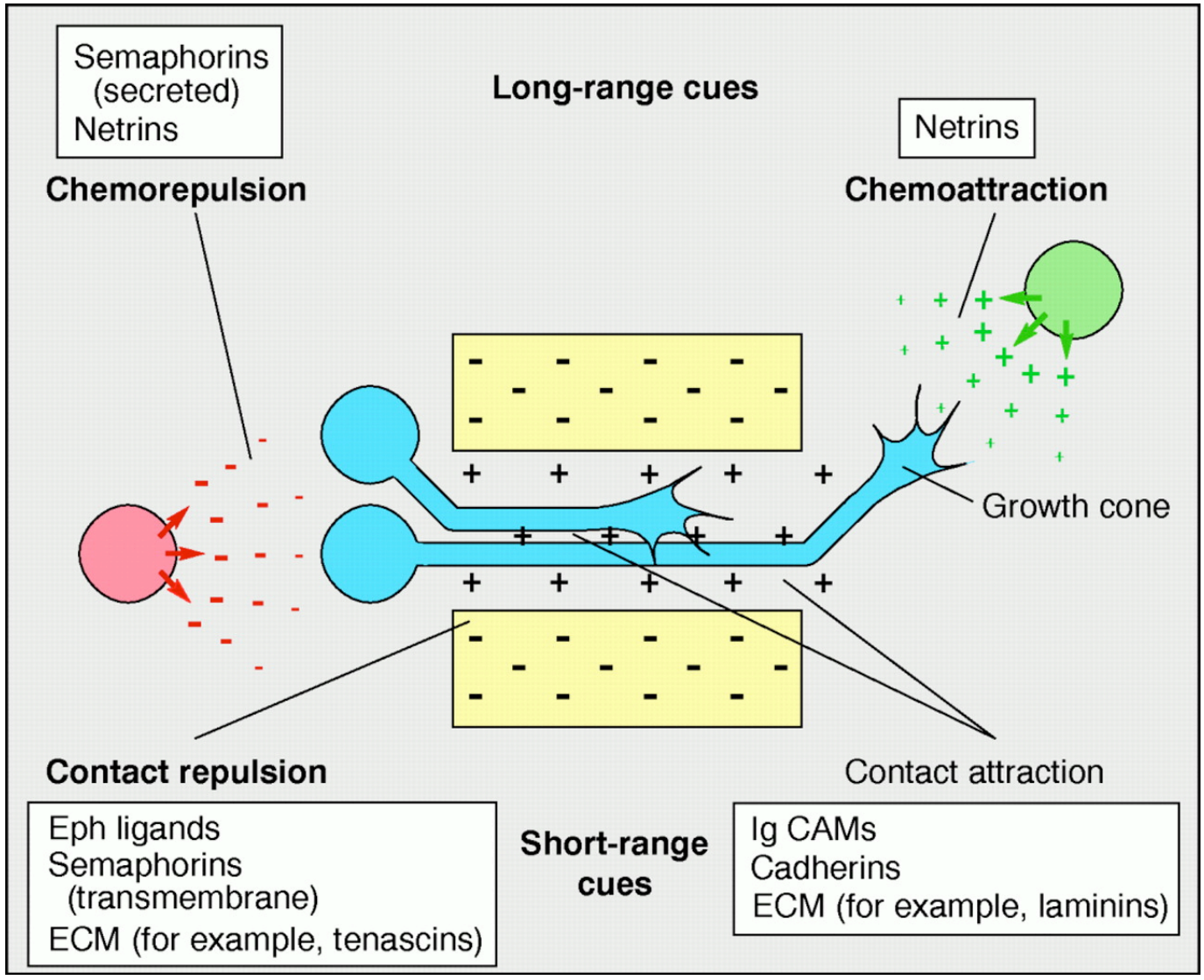
Last time: axon guidance cues/receptors

- Axons are guided to target region by extracellular guidance cues which interact with axonal receptors
- Guidance cues can be diffusible or membrane-bound, act locally or over a distance
- A given guidance cue can trigger attraction or repulsion --- depends on :
  - Repertoire of guidance receptors
  - Others cues received
  - State of signaling pathways in growth cone

Reaching the target region

- Axons usually pass through intermediate targets to reach final target
- Navigational decisions involve the integration of multiple signals in an axon-specific fashion :
  - Example: regulation of projection to and across the midline via Netrin, Slit, Robos and Comm

Major navigational forces



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Navigation in the target region

- **Axon reaches target region: still many possible target cells: How does axon choose correct one?**
  - **Topographic map formation (reach appropriate location within target field)**
  - **Post-synaptic target cell selection**

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Topographic maps

- Orderly anatomical representations of a physical property of the world (visual space, sound frequency, odor)
- Basic types:
  - Ordered by anatomical position (eg. visual system)
    - » adjacent neurons project to adjacent targets
  - Ordered by neuron type (eg. olfactory system)
    - » neuron expressing same odorant receptor (detecting same odor) project to same place

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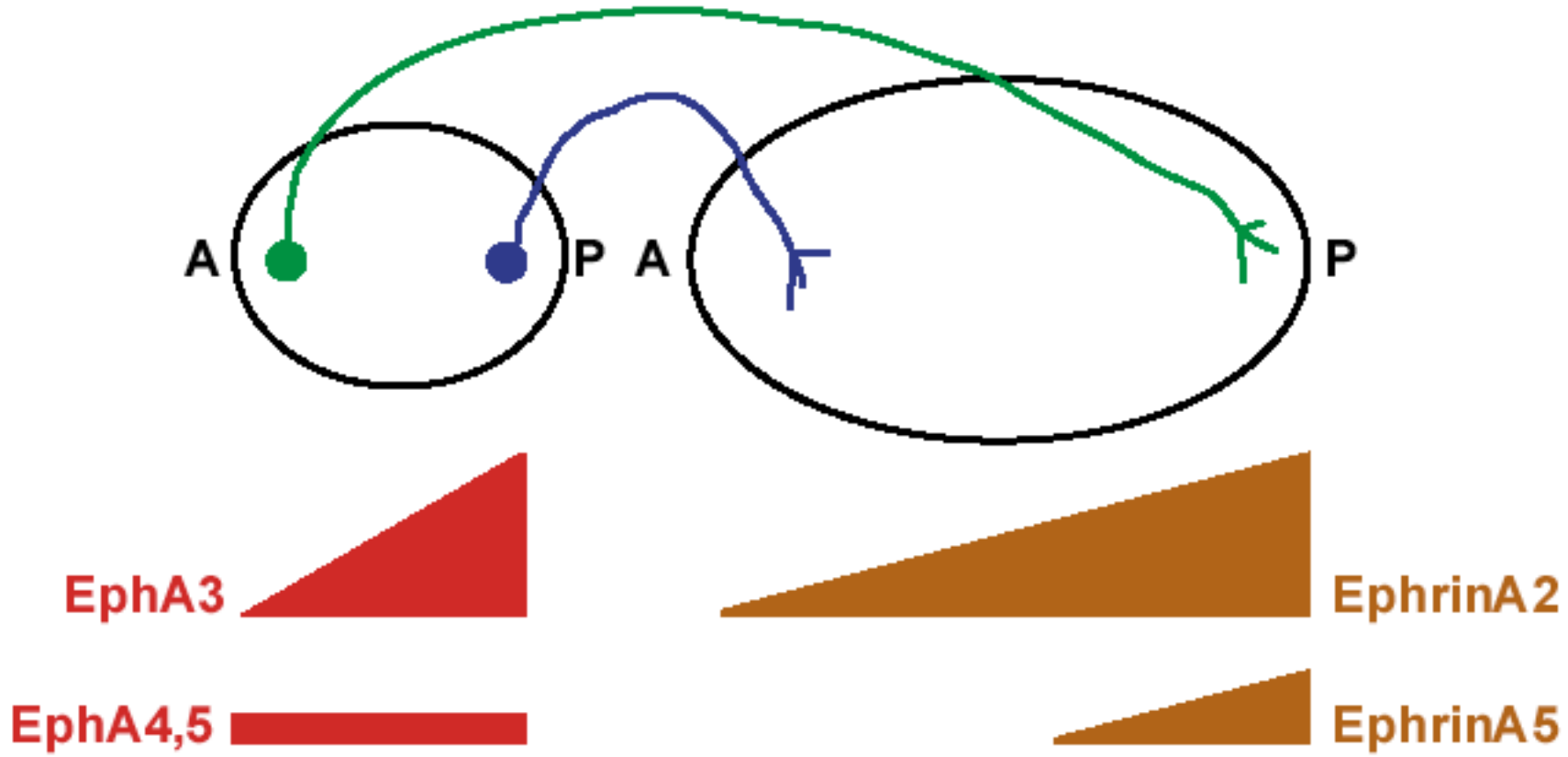
Eph and Ephrins in the Retinotectal System



- Expressed in complementary gradients on axons/targets.

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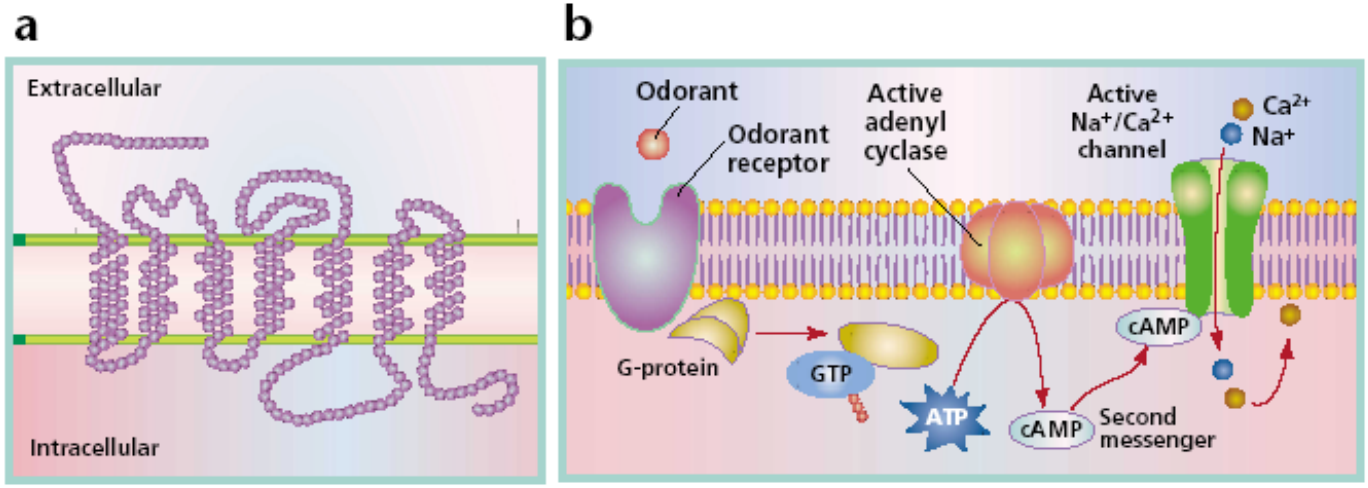
Mammalian olfaction

- Mice: ≈1500 olfactory receptor genes (7-TM receptors)
- Humans: ≈1000 olfactory receptors (≈2/3 psuedo-genes)



Olfactory receptor gene expression

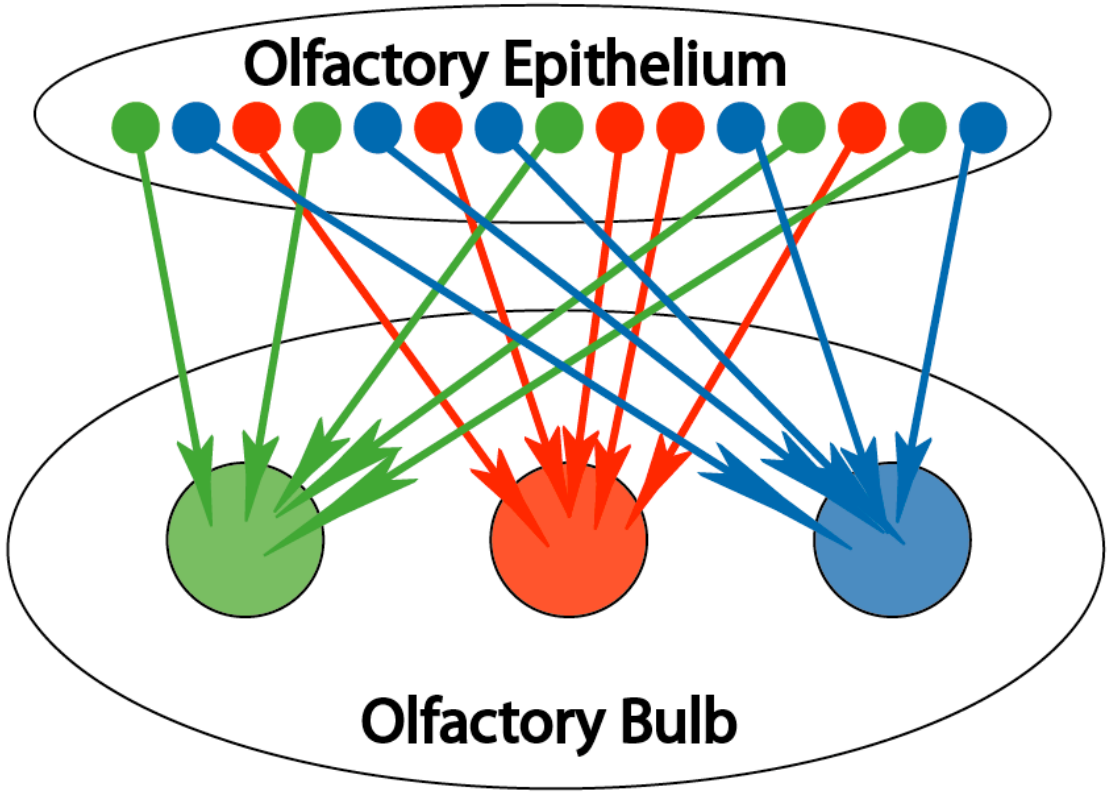
- 1 Olfactory receptor per olfactory neuron
- Diploid: 2 alleles of each olfactory receptor locus ---only 1 of the 2 loci is expressed (monoallelic expression)
- Analogous to expression of B and T cell receptors -- single B or T cell receptor expressed via DNA rearrangement
- Recent work suggests olfactory receptor expression does not involve irreversible alterations to DNA:

  Take nucleus from differentiated olfactory neuron and use to clone a whole mouse --- this mouse has apparently normal diversity of OR expression
- How an individual OR expresses only a single allele of a single OR is unknown

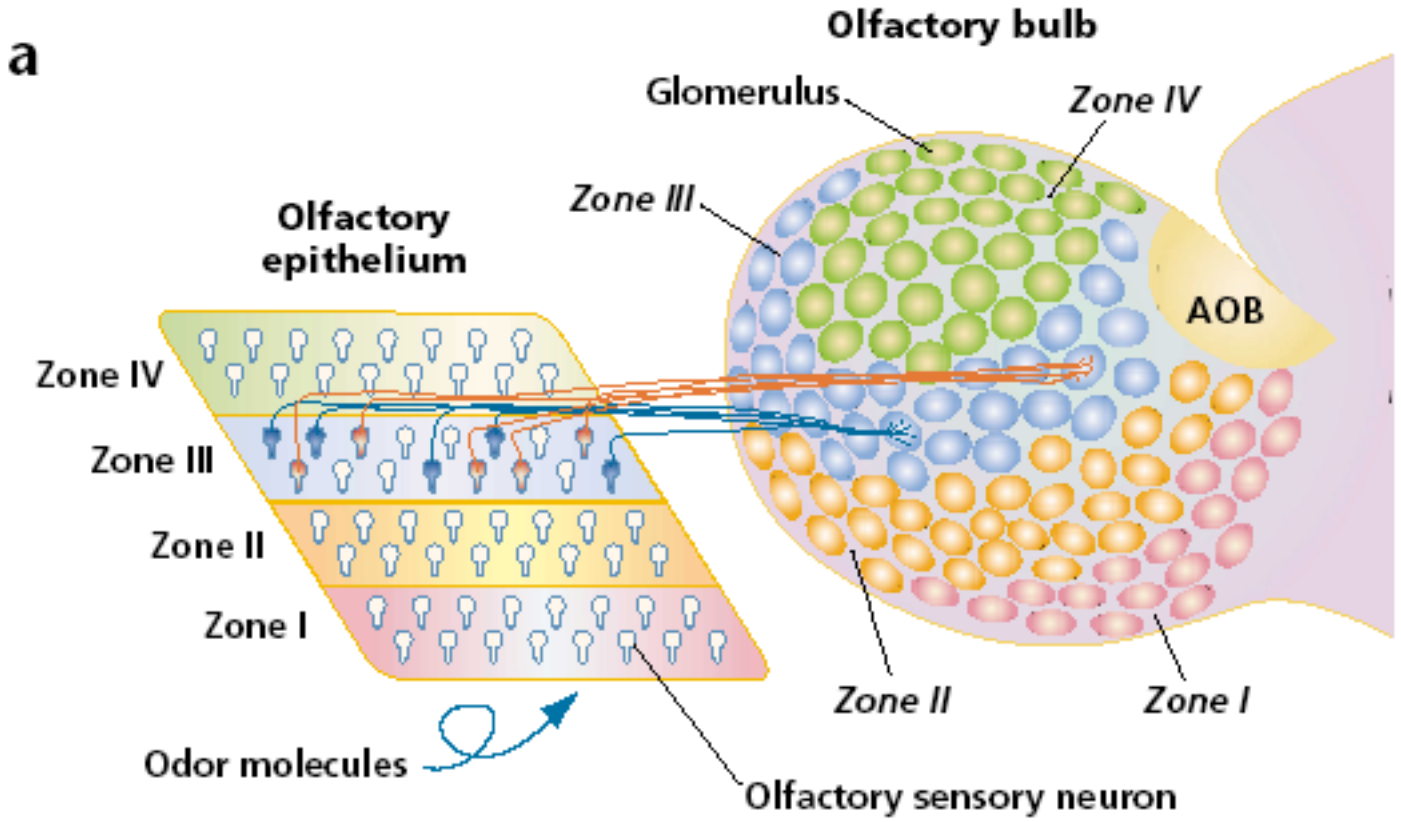
Wiring of the Olfactory System

- Over a thousand different types of olfactory neurons (each type expresses a particular receptor)
- Problem of odor distinction requires determining which olfactory neurons are activated
- How tell which olfactory neurons are activated?

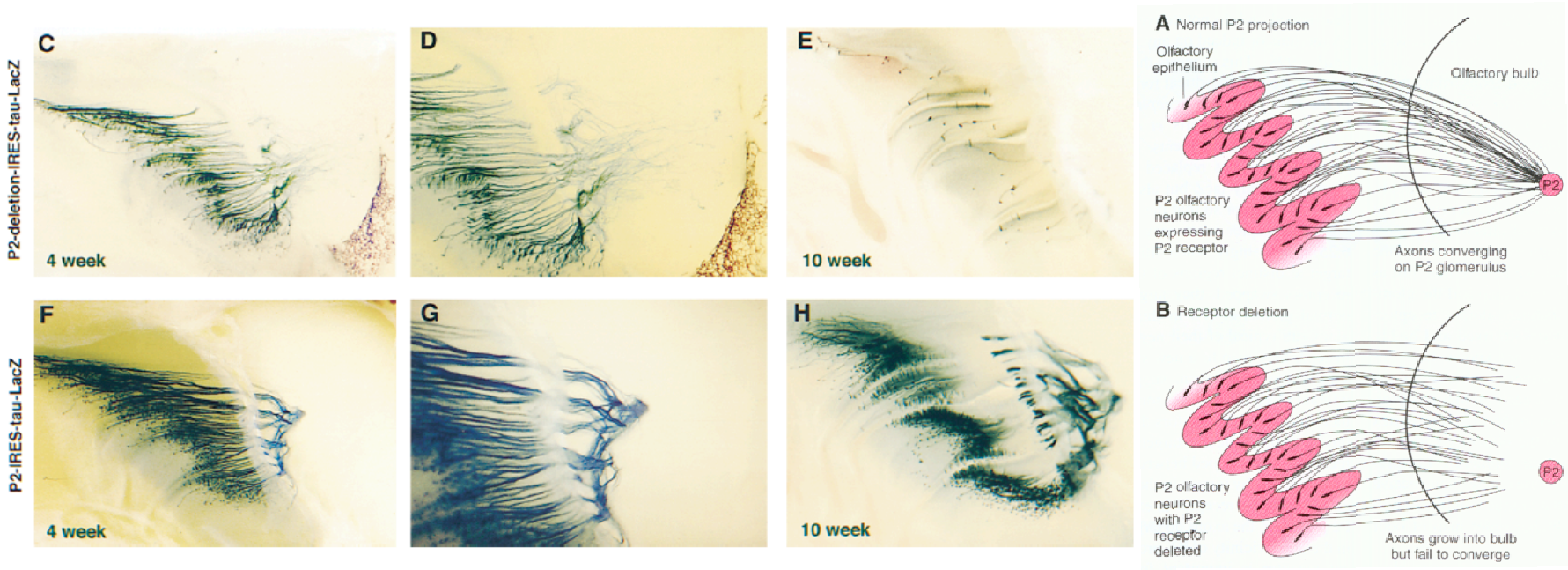
Olfactory axons expressing the same receptor project to the same target in the olfactory bulb



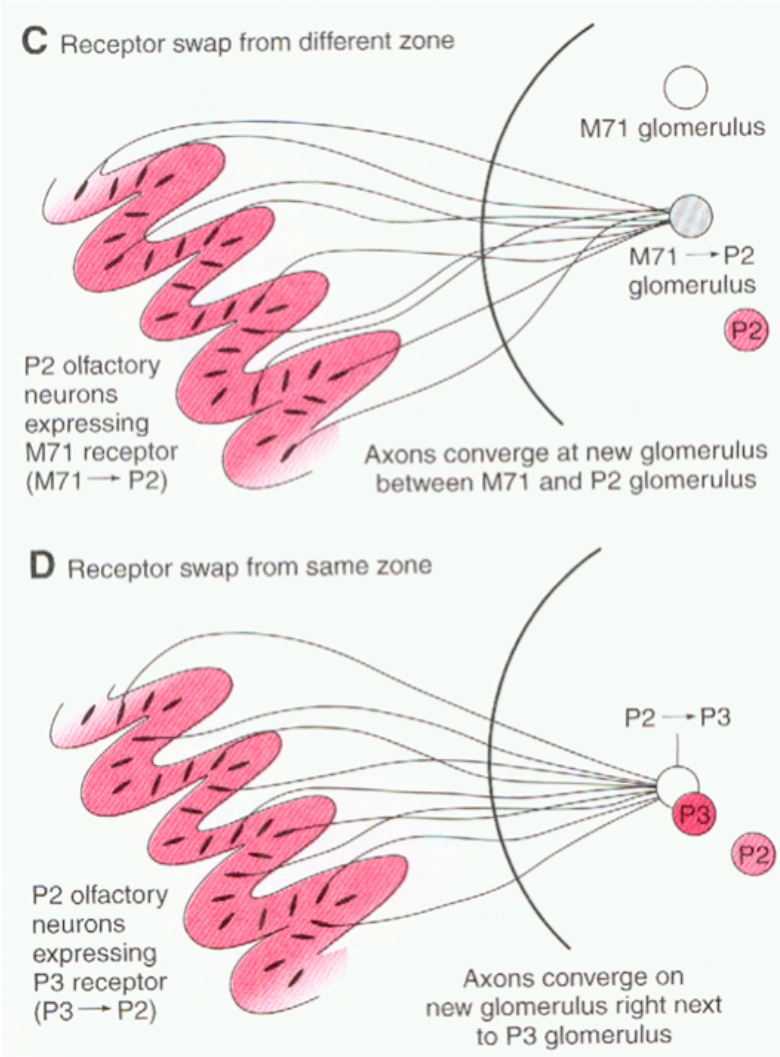
Olfactory receptor expression is not entirely random: epithelium segregated into zones



Targeting of olfactory axons depends on olfactory receptor expression



Olfactory axon target specificity depends on identity of olfactory receptor

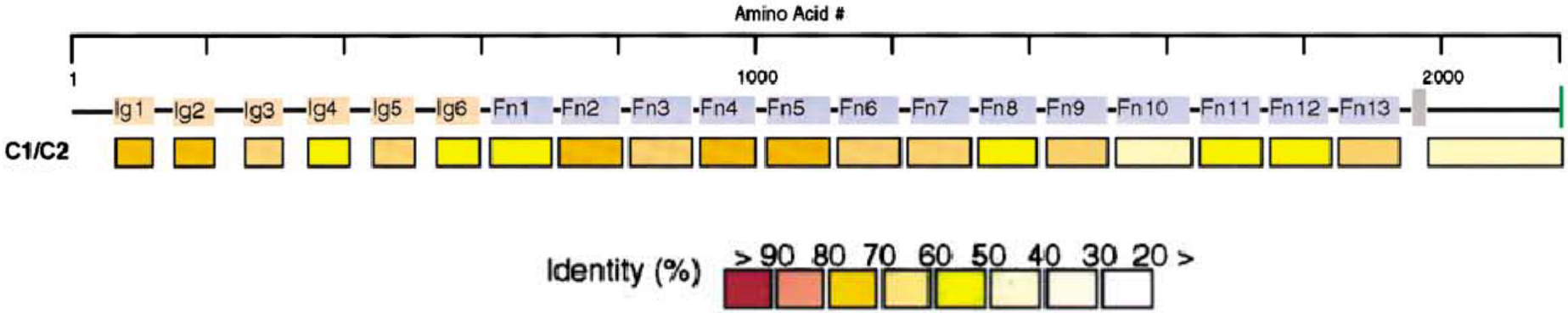


Olfactory axon map formation

- Target selection specificity involves olfactory receptor
- Role of olfactory receptor unclear:
  - traditional guidance receptor? if so, what are the cues?
  - activity-dependent mechanisms enforcing specificity? "fire together, wire together"
- Other cues may interact to help establish zonal projection pattern (Eph/ephrin gradients)
- Similar kind of convergence observed in fly olfactory system -- ≈50 glomeruli; receptor-independent

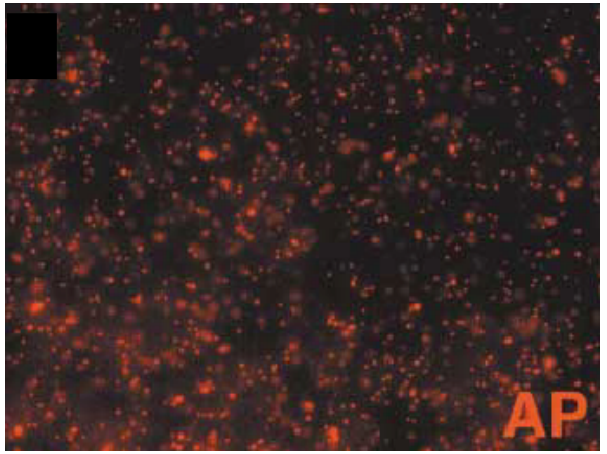
Cell-cell recognition molecules implicated in target cell selection in the retina

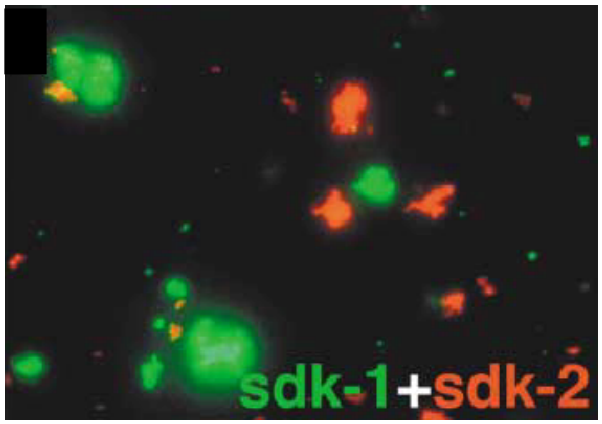
- Sidekicks: Transmembrane members of the Immunoglobulin superfamily (IgSF) : 6 Igs, 13 Fns
- Chickens have two Sidekicks: Sdk-1, Sdk-2 (59% identical)



Sidekicks (Sdks) are homophilic cell adhesion molecules (CAMs)

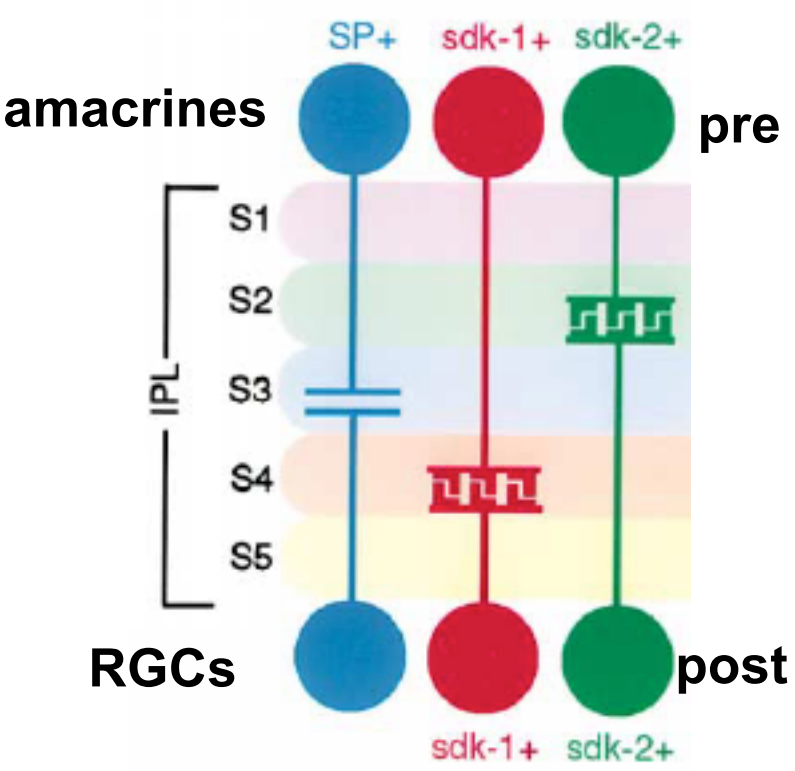
- Sdk's act as homophilic cell adhesion molecules
  - » Homophilic: interact with the same protein on adjacent cell
  - » Heterophilic: interact with different protein on adjacent cell
- Sdk1 binds Sdk1
- Sdk2 binds Sdk2
- Sdk1 and Sdk2 do not associate





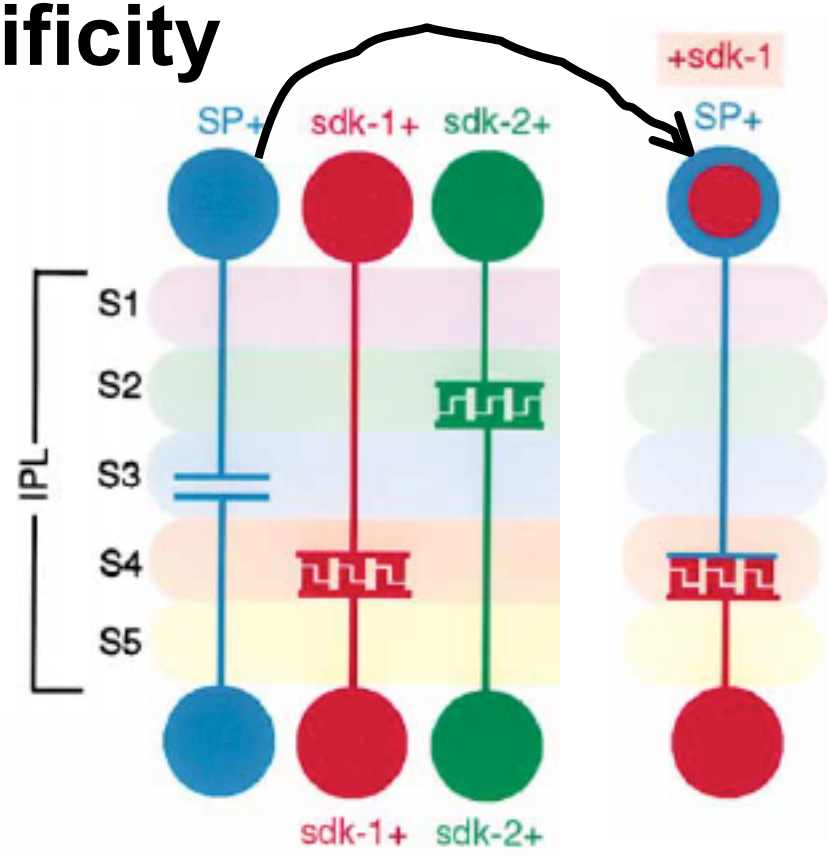
Sidekicks in axon/dendrite recognition in the chick retina

- Sdk-1 and Sdk-2 are expressed by distinct subsets of Retinal Ganglion Cells (RGCs) (each in ≈25% of RGCs) and pre-synaptic inputs of RGCs (amacrine cells, bipolar cells)
- Evidence suggests:
  - Sdk-1$^+$ amacrine/bipolar axons contact Sdk-1$^+$ RGC dendrites
  - Sdk-2$^+$ amacrine/bipolar axons contact Sdk-2$^+$ RGC dendrites
- Sdks could allow specific subsets of axons and targets to recognize one another



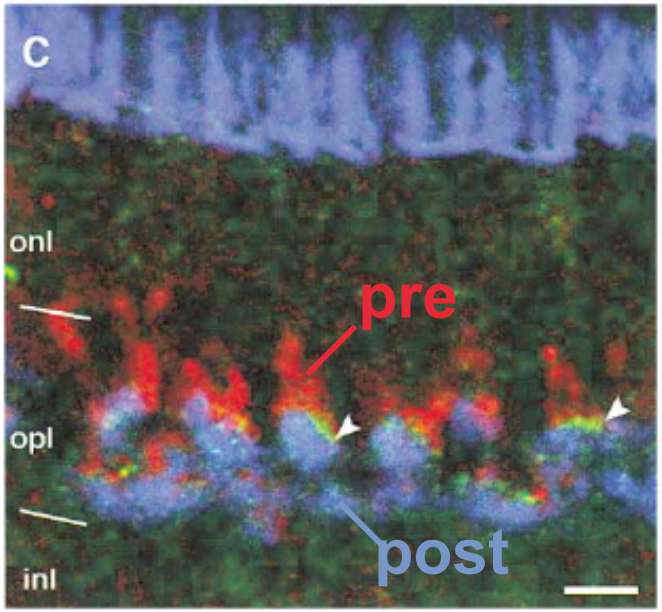
Sidekicks can alter axon/target specificity

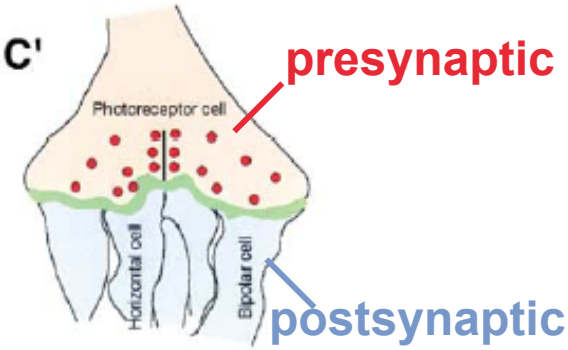
- Express Sdk-1 in Sdk-negative amacrine cells
  - axons retarget to the Sdk1^{+}-layer (figure)
- Express Sdk-1 in Sdk-negative RGCs
  - dendrites retarget to the Sdk1^{+}-layer
- Also true for Sdk-2
- Thus, Sdk expression can regulate target selection



Sidekicks in target specificity

- Differential Sdk expression may underlie differential target recognition in the retina (no loss-of-function yet)
- Sdks localize to synaptic sites --- could assist assembly of pre/post-synaptic sites
- Sdks also have intracellular domains ---Sdks likely act not only through surface adhesion, but by coupling to cytoskeleton and intracellular signaling pathways

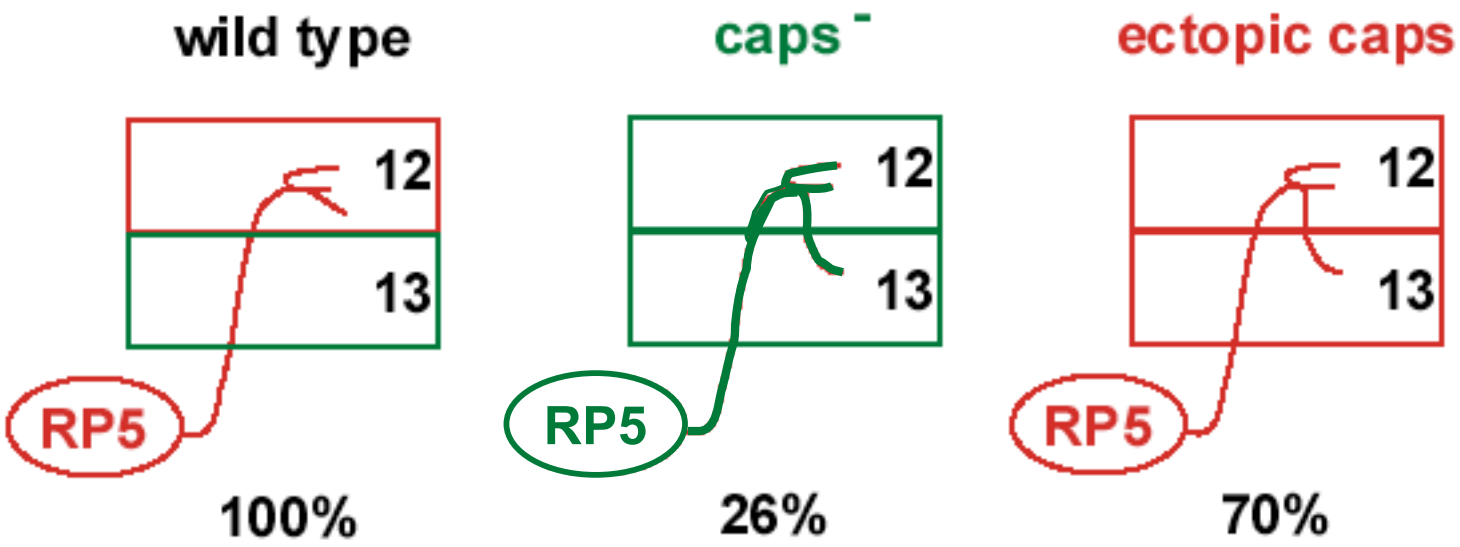




Capricious: a transmembrane protein important for target selection

- **Doesn't act as homophilic CAM.**
- **Expressed on ~1/3 of *Drosophila* body wall muscles.**
- **Expressed on axons that innervate these muscles.**

Capricious: target selection molecule in *Drosophila* motor axon/muscle system

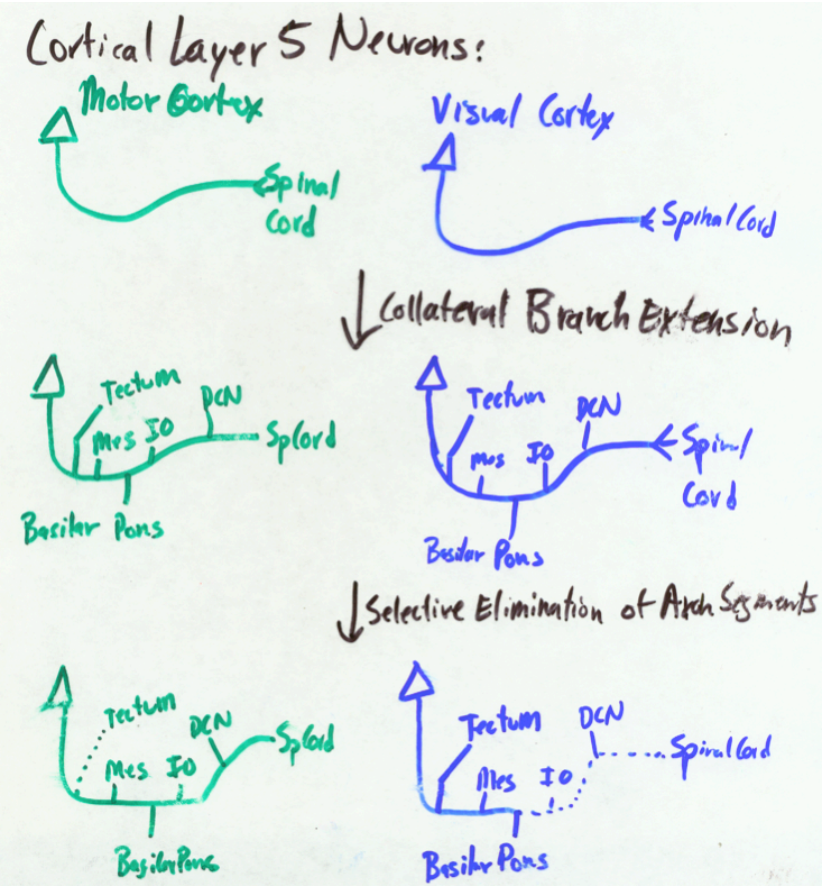


- Necessary and sufficient for target selection (sort of..)
- Effects are partial
- Expressed on a large fraction of neurons and targets

Probably part of a combinatorial code.

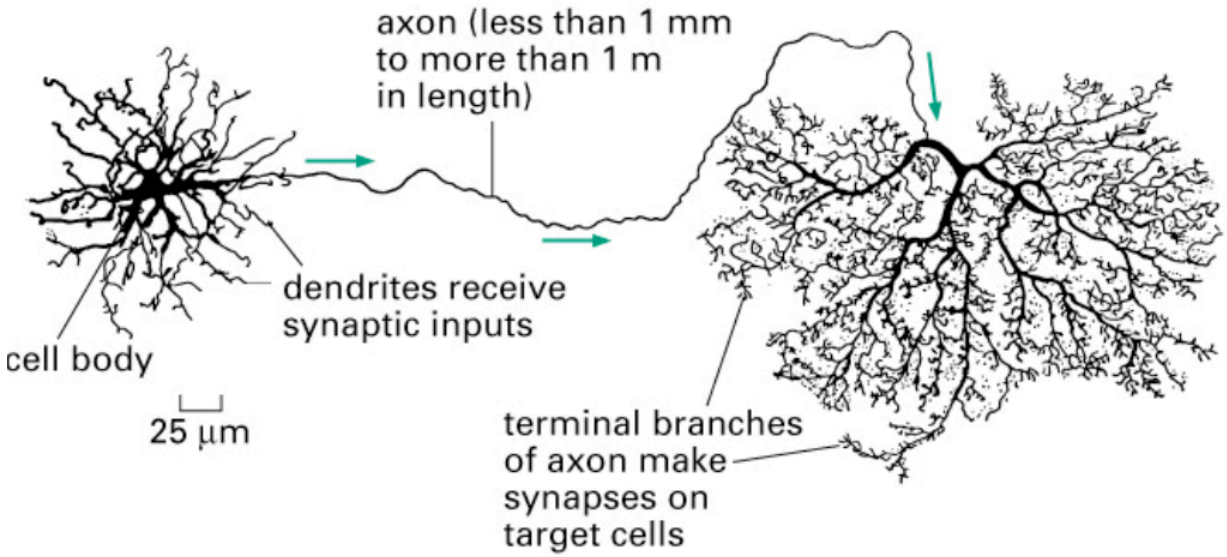
Collateral branching and selective retention: Axon targeting can involve remodeling

- Layer 5 neurons initially contact multiple target regions
- Neurons from different areas of cortex contact similar target regions
- Final projection patterns established through selective elimination of axon segments



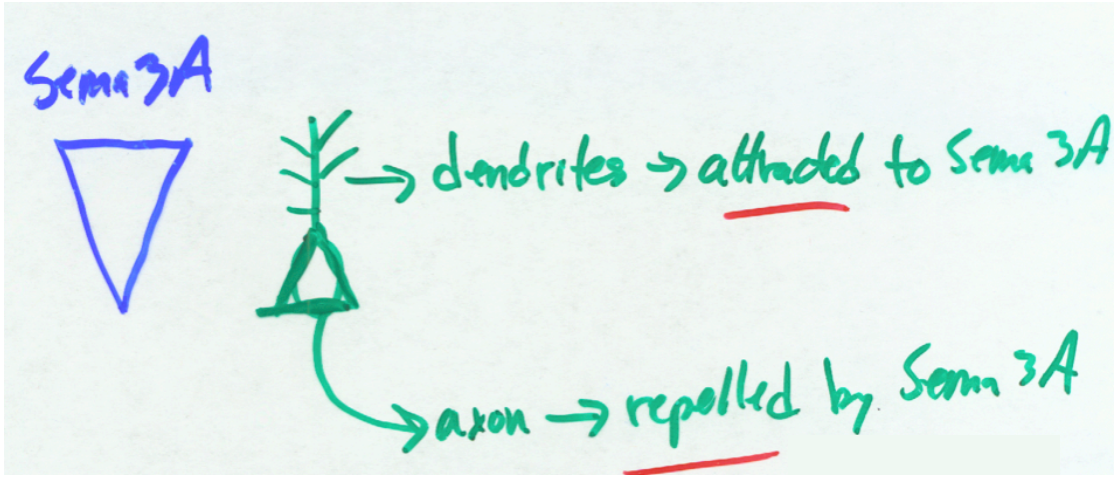
Dendrite guidance

- A neuron's axons and dendrites project to different targets
- How can different projections from a single cell choose distinct targets?
- Do different cues/receptors guide dendrites?
  - No -- axon guidance cues/receptors also guide dendrites
- How the same cue affect axons and dendrites differently?



A neuron's axons and dendrites can respond differently to the same signal

- Cortical neurons respond to the secreted cue Sema3A
- Sema3A is present in a graded fashion (highest apically)
- Axons and dendrites both respond to Sema3A, but differently
  - Axons repelled, dendrites attracted



How do the axons and dendrites respond differently to Sema3A?

- Different receptors? No
- How generate different responses in same cell?
- Recall: cyclic nucleotide levels and additional cues can switch a growth cone's response to a cue (Netrin, cAMP, laminin example)
- Sema3A response is sensitive to cGMP levels in *in vitro* turning assays:
  - High levels of cGMP : Sema3A is attractive
  - Low levels of cGMP : Sema3A is repulsive

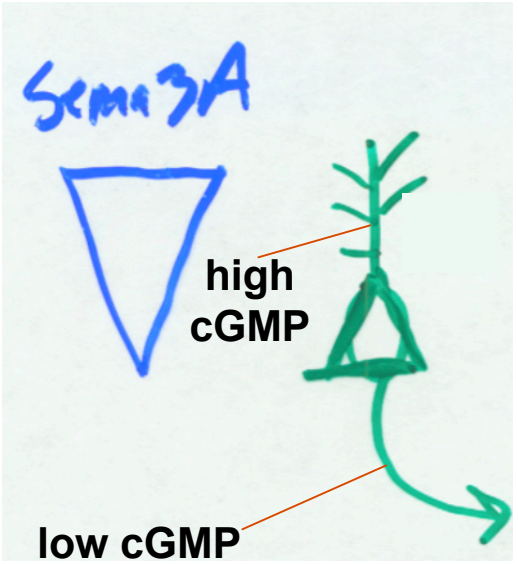
Nature of response to Sema3A affected by cGMP signaling

- Soluble guanylyl cyclase (sGC) produces cGMP
- sGC is concentrated in cell body and dendrite; not present in axon
- Suggests:
  - higher levels of cGMP in dendrite (attraction)
  - lower levels of cGMP in axon (repulsion)

sGC

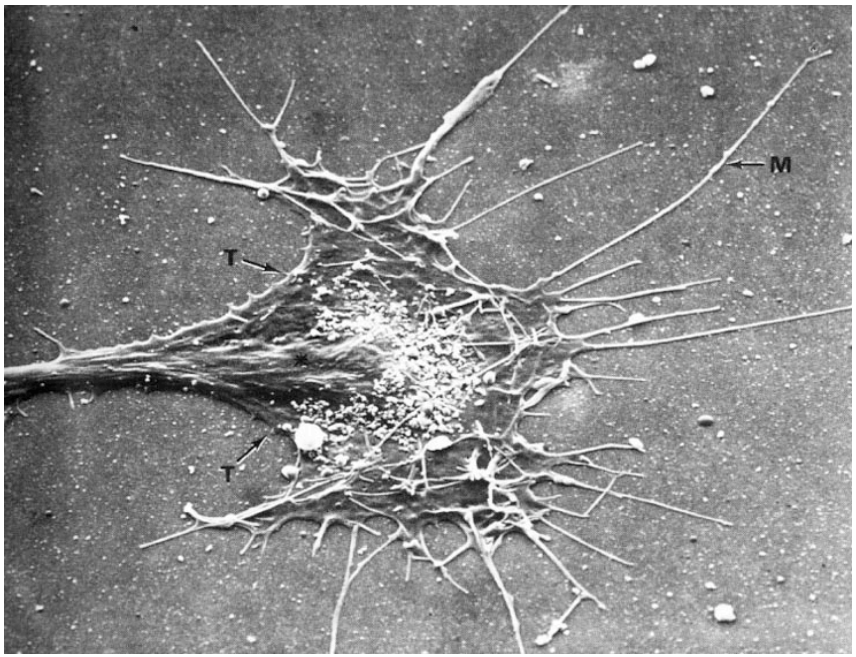
Nature of response to Sema3A affected by cGMP signaling

- Inhibit sGC or Protein Kinase G (cGMP-regulated kinase):
  - No effect on axon
  - Apical dendrites grow randomly
- Consistent with:
  - High cGMP dendrite ---attracted by Sema3A
  - Low cGMP axon --- repelled by Sema3A
- Repertoire of downstream signaling molecules appears responsible for differential response to Sema3A

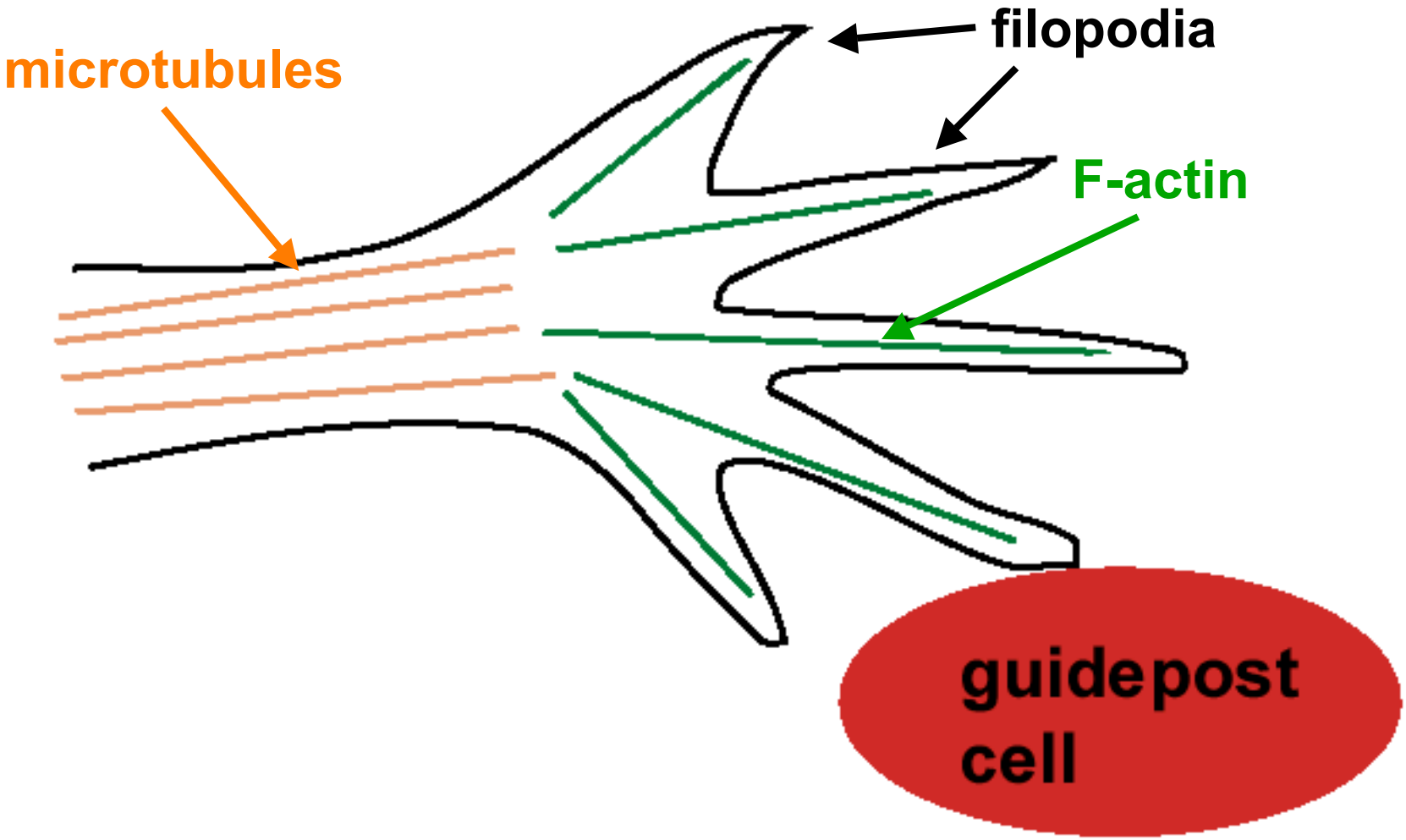


The growth cone

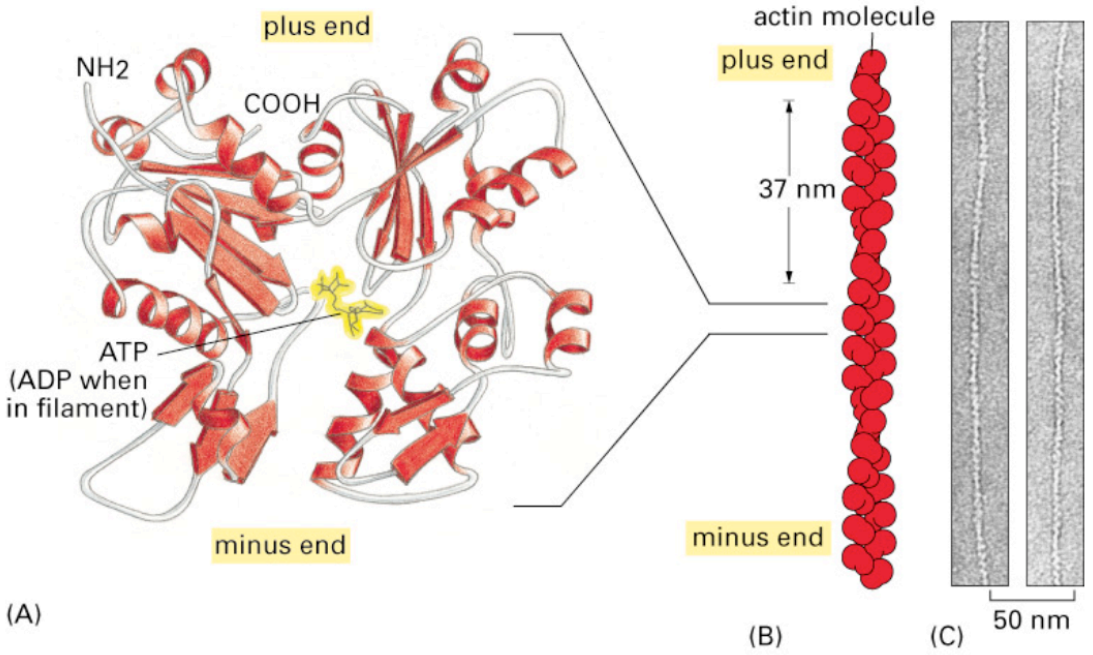
- Structure at leading edge responsible for navigation
- Responds to cues within minutes.
- Can continue to navigate (for a while...) if severed from cell body.



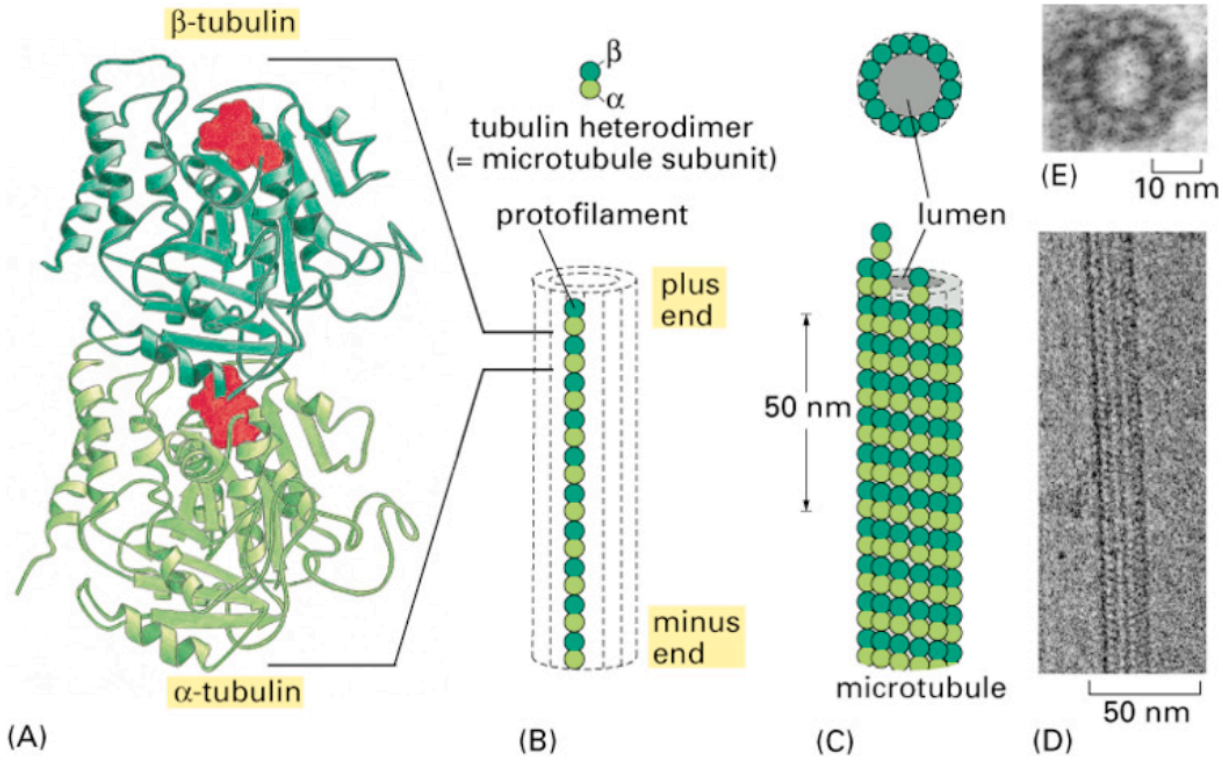
The growth cone cytoskeleton



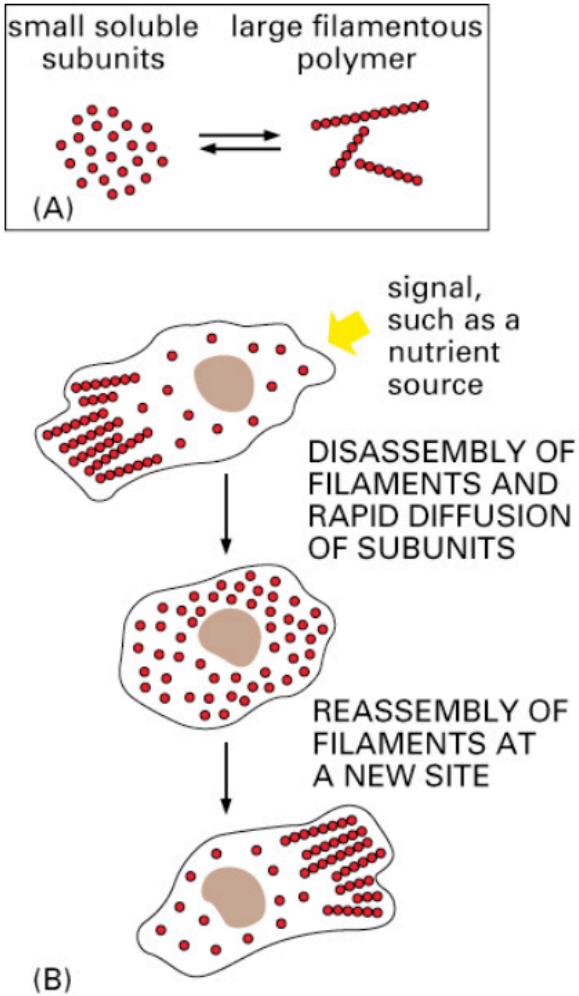
Actin


plus end
NH2
COOH
ATP
(ADP when
in filament)
minus end
(A)
actin molecule
plus end
37 nm
minus end
(B)
(C)
50 nm

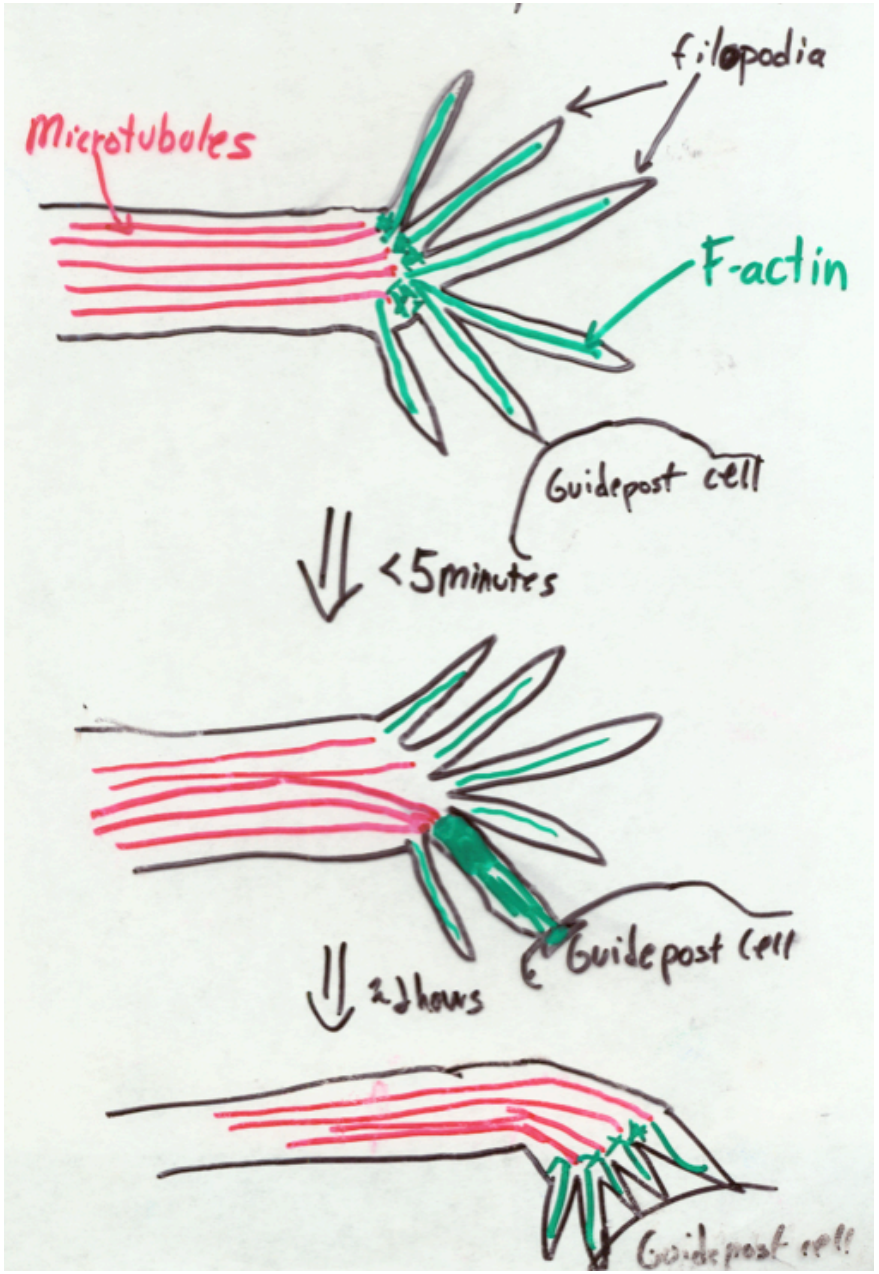
Microtubules



Cytoskeletal changes underlie cell movements



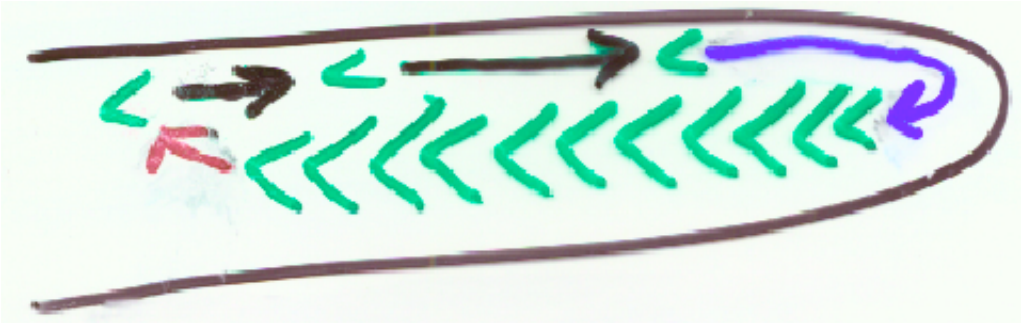
The growth cone cytoskeleton is both highly structured and dynamic



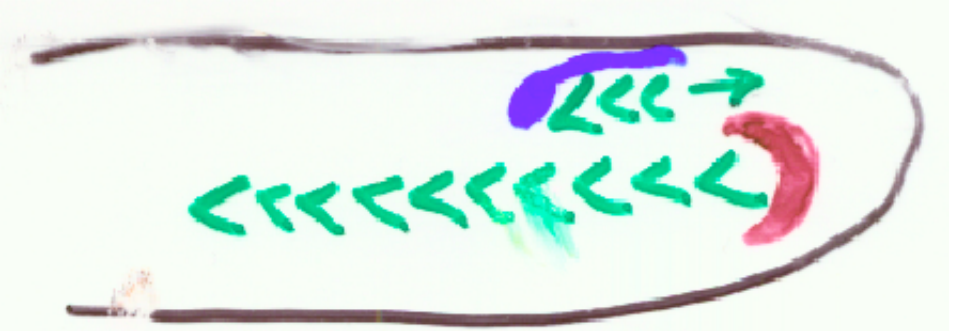
Actin dynamics suggest two levels for the control of growth cone motility by signals

1. Filament assembly/disassembly

-polymerization/depolymerization



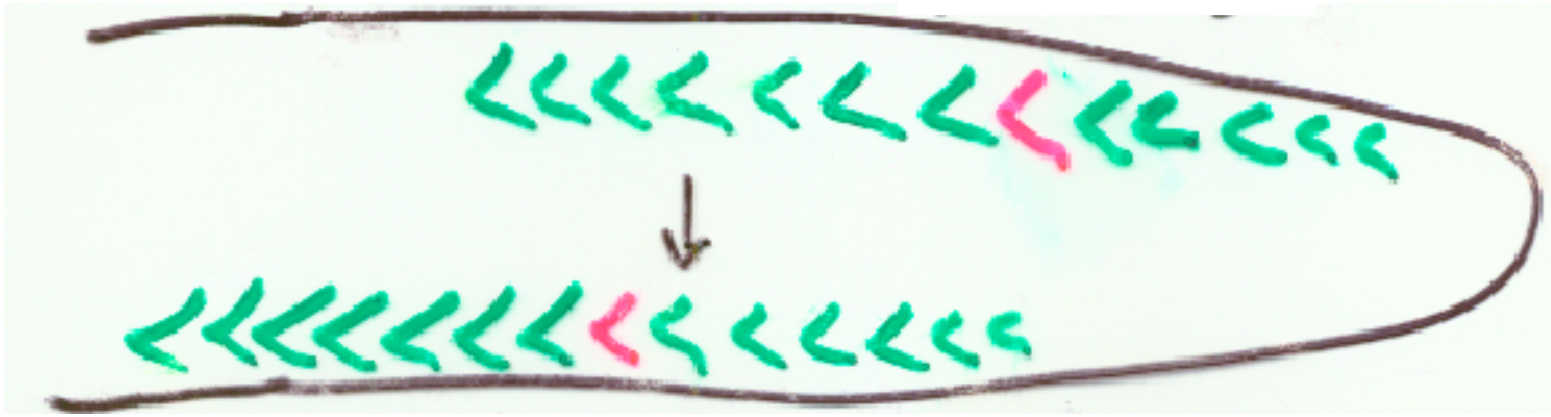
-filament nucleation and filament capping



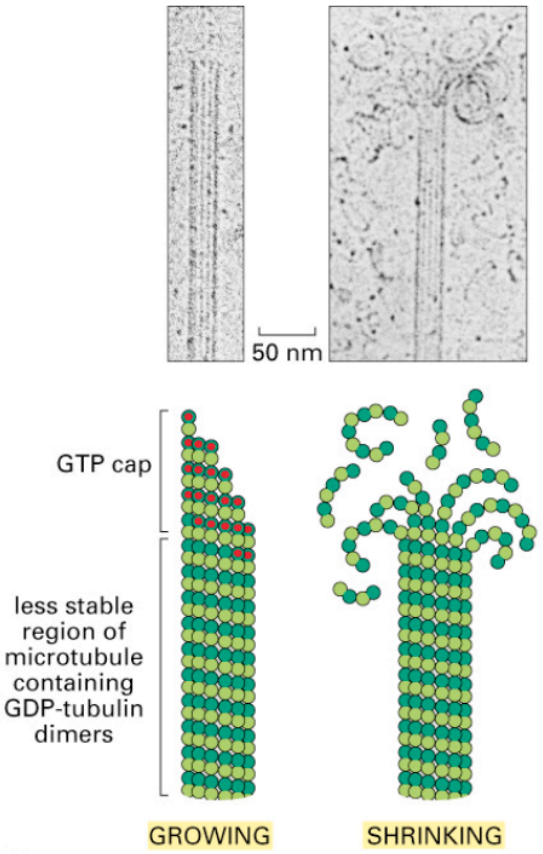
Actin dynamics suggest two levels for the control of growth cone motility by signals

2. Retrograde flow

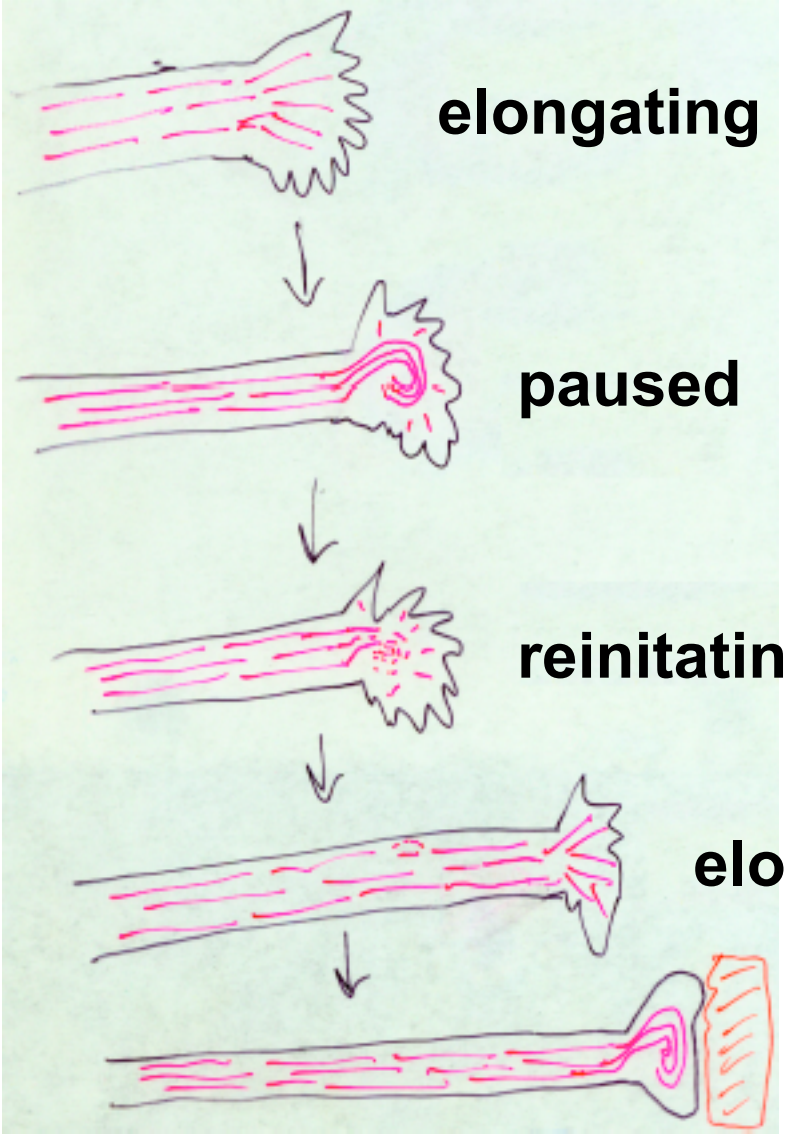
- myosin motor driven
- counteracted by coupling to substrate?



Microtubule dynamics



Microtubule organization is also highly dynamic and regulated



How do guidance receptors regulate cytoskeletal structure?

- Rho-family GTPases are important targets for relaying information from receptors to the actin cytoskeleton.

- 1992: Ridley & Hall; 1994: Nobes & Hall

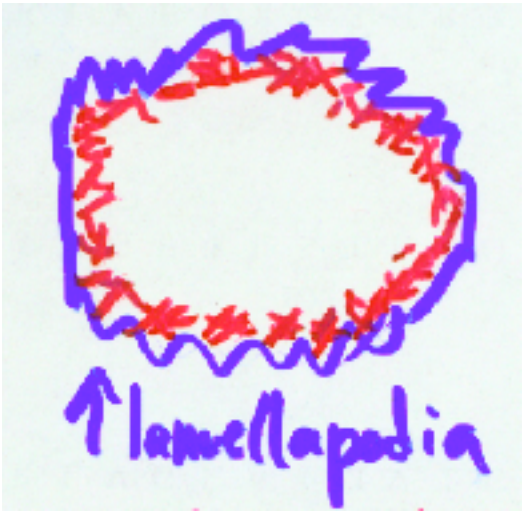
  injected activated Rho GTPases into fibroblasts
  --got dramatic reorganization of actin cytoskeleton within minutes!!

Injection of activated GTPases into fibroblasts

activated Rho

actin fibers
at focal adhesions

activated Rac



actin meshwork
at leading edge

activated cdc42

actin bundles

- Rho family GTPases are powerful regulators of the actin cytoskeleton.
- Different family members can have different effects

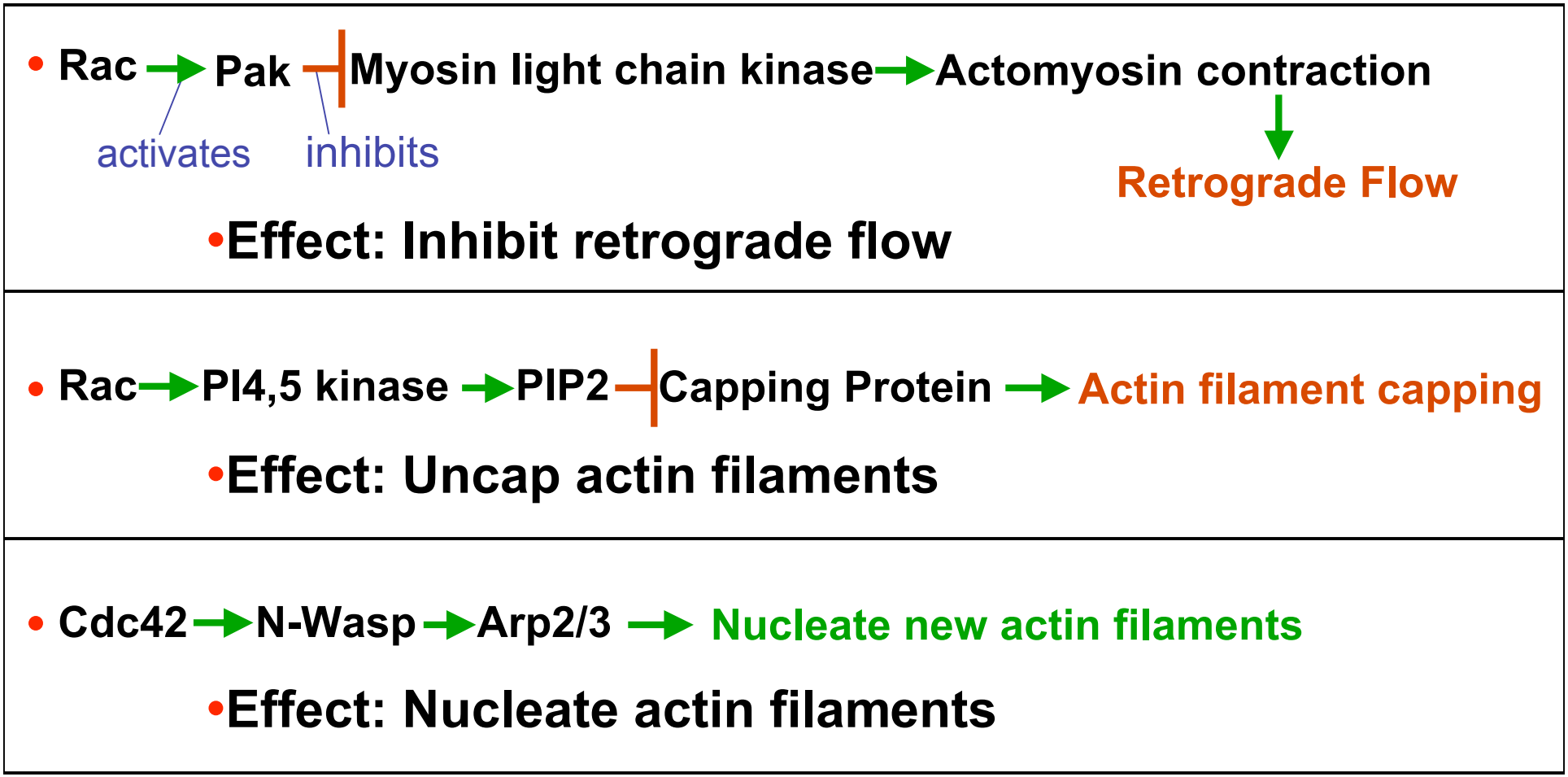
RhoGTPases influence actin dynamics at many different steps

- **Actin dynamics:**
- **nucleation of new actin filaments**
- **polymerization/depolymerization of existing filaments (capping/uncapping of filament ends)**
- **retrograde flow of actin filaments (myosin-dependent)**

Rho GTPases work through effectors

- Rho GTPases regulate protein and lipid kinases, scaffolding proteins…
- Different Rho GTPases cause different changes in cell structure and movement because they regulate different sets of effectors

Possible routes from Rho-family GTPases to the cytoskeleton



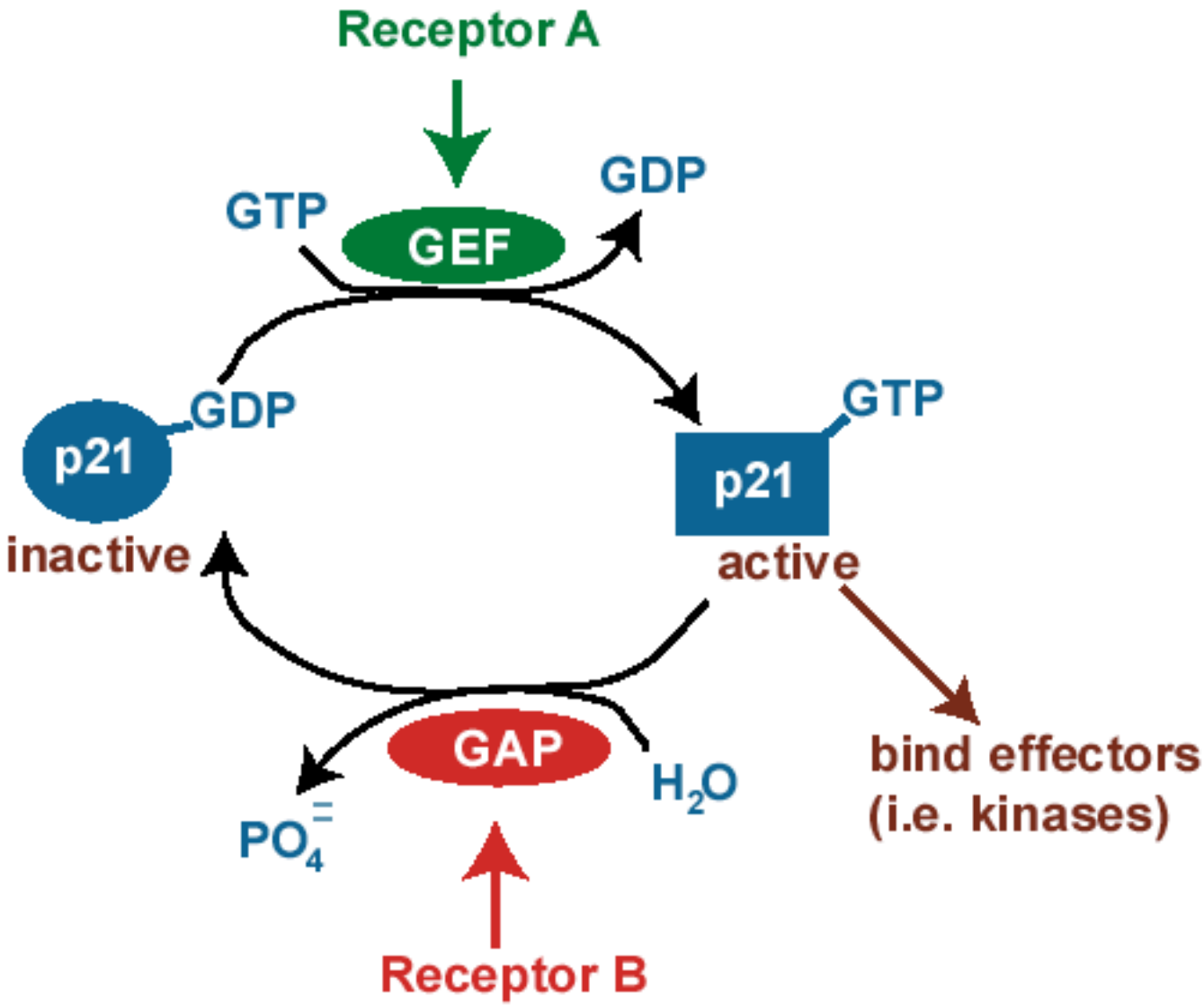
Rho family GTPases act as molecular switches

- members of the ras superfamily of p21 GTPases
- subject to both positive and negative regulation by:

  - guanine nucleotide exchange factors (GEFs) (positive)

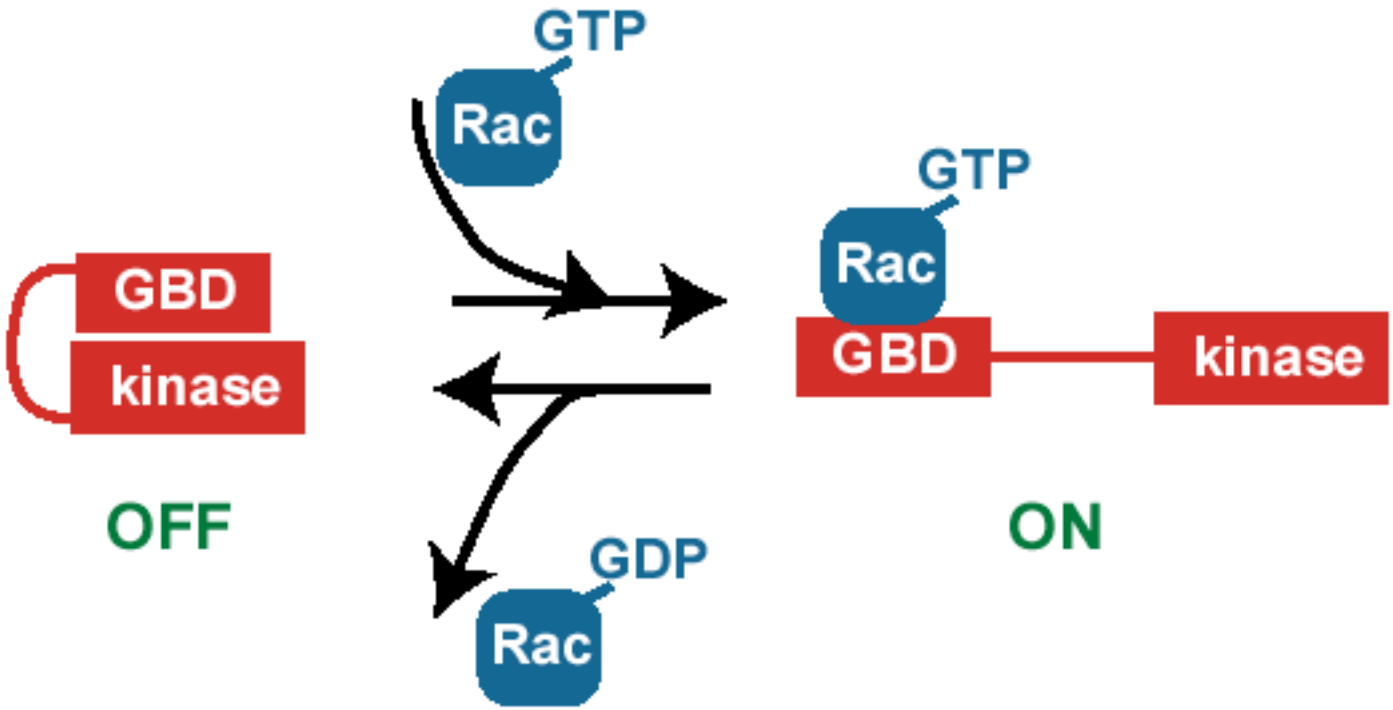
  - GTPase activating proteins (GAPs) (negative)

The p21 GTPase cycle



How can putting a Rho-family GTPase into the GTP-bound regulate an effector?

- One example: PAK (a serine/threonine protein kinase) is activated by binding to Rac^{GTP} and $cdc42^{GTP}$



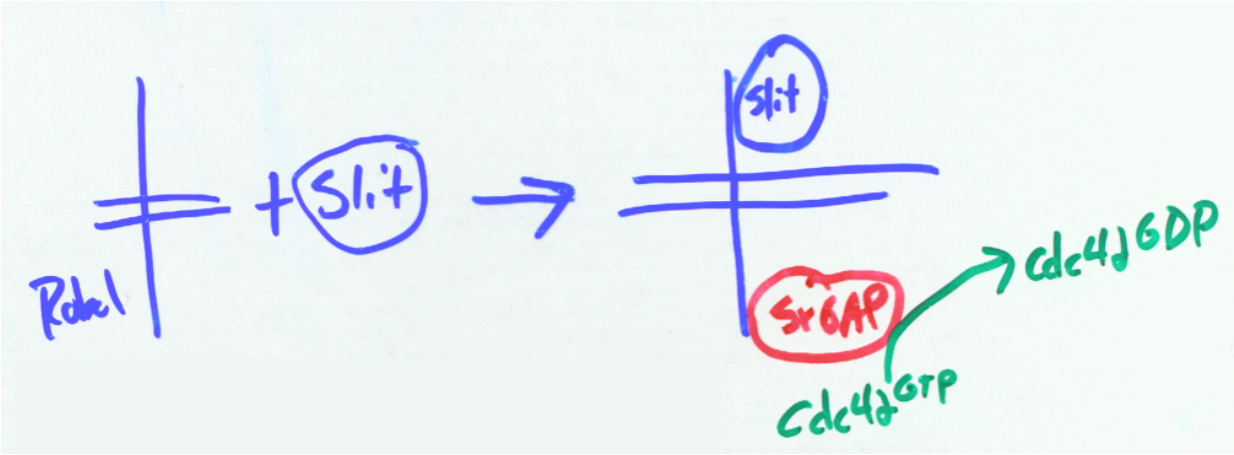
- Crystallographic and biochemical data suggest this type of activation mechanism may be used in many RhoGTPase-effector interactions.

Rho GTPases in neural development

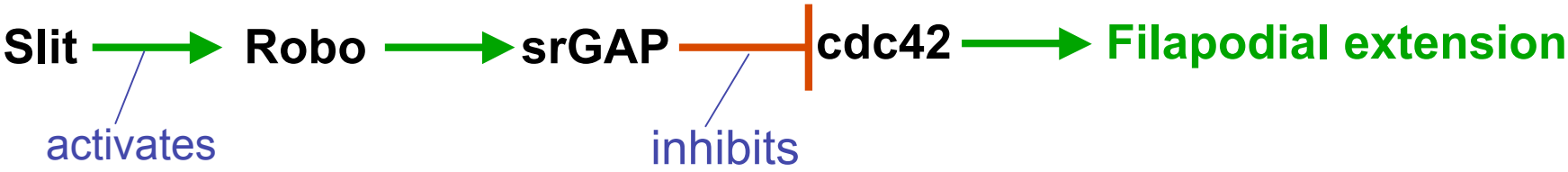
- Mutations in Rho-family members disrupt axon guidance (and other aspects of neuronal morphology)
- Mutations in Rho-family regulators and effectors are found in a number of mental retardation syndromes in humans
  - MRX46: Rac/Cdc42 GEF
  - MRX 60: Rho-family GAP
  - MRX30, MRX47: Pak3 (mutations in GBD)

Rho family GTPases are important targets of axon guidance receptors

- Robo signals through a GAP for cdc42 called srGAP
- Slit/Robo response blocked by interfering with srGAP function or using a mutant form of cdc42 locked into the GTP state

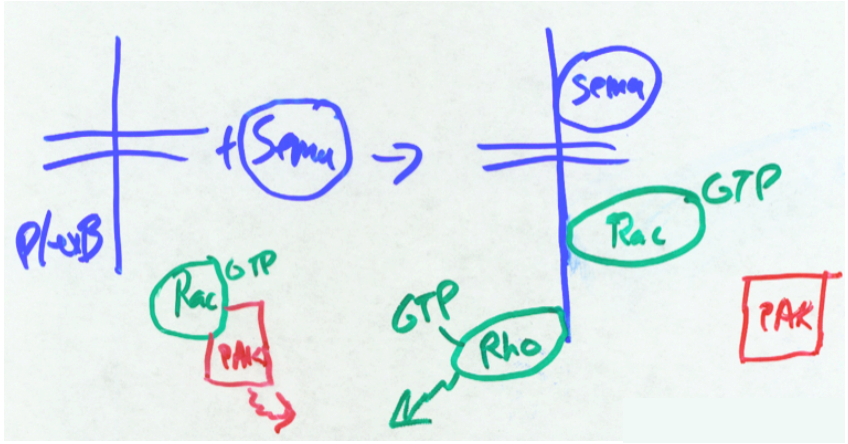


- Consistent with this type of pathway for Slit/Robo repulsion:

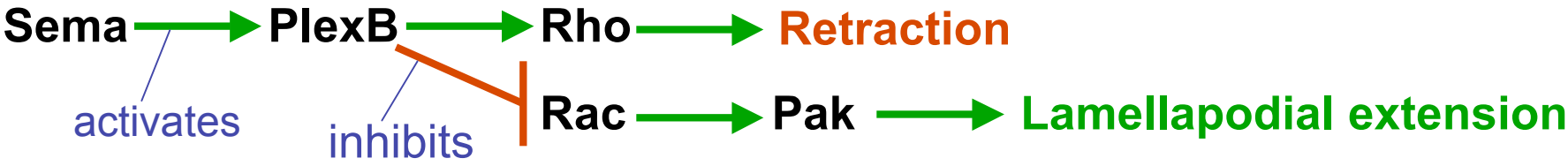


Rho family GTPases are important targets of axon guidance receptors

- The PlexB guidance receptor for Semaphorin family cues binds directly to Rho GTPases



- Sema binding to PlexB stimulates signaling by Rho and inhibits signaling by Rac (prevents binding to Pak)
- Consistent with this type of pathway for Sema/PlexB repulsion:



Guidance receptor signaling to the cytoskeleton

- Guidance receptors interact with Rho GTPases and their regulators to generate the cytoskeletal changes responsible for guidance
- However: Rho GTPases are not the whole story: there are additional routes from receptors to the cytoskeleton
  - For example: Mena proteins bind to guidance receptors and directly to actin filaments

Responding to chemotropic gradients

- Axons need to be able to sense and grow toward or away from sources of diffusible cues
- How sense and response to potentially shallow gradients of cues?
- Detailed molecular mechanisms in growth cone not yet known
  - Appears to involve repeated cycles of sensitization/desensitization (axons zig-zag)
- Molecular mechanisms beginning to be worked out in leukocytes and in Dictyostelium discoideum (slime mold)

Visualizing cytoskeletal changes in Dicty cells sensing chemoattractant

http://dictybase.org/tutorial/gerisch1.avi

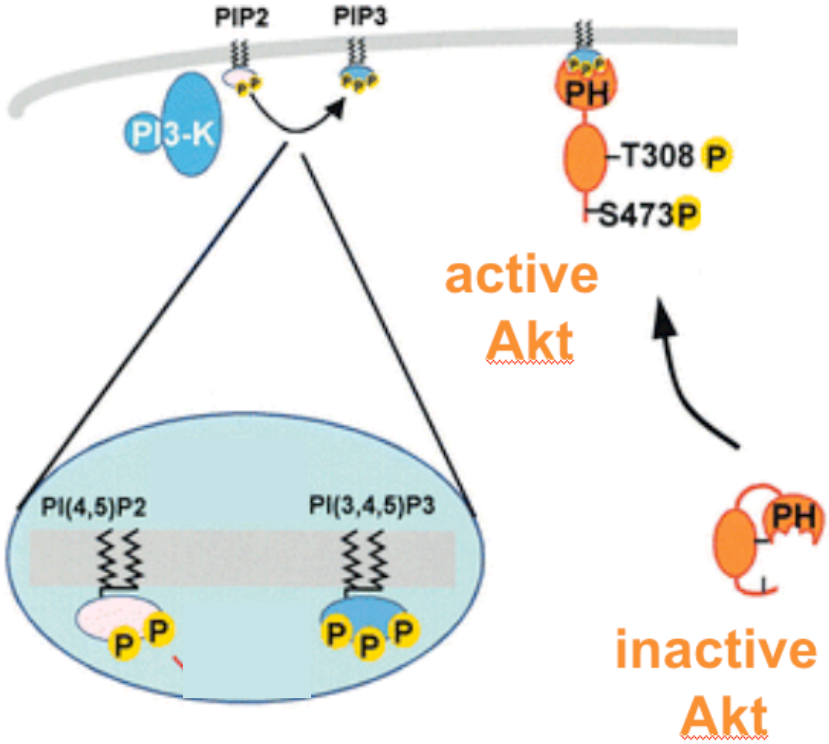
- **GFP-Actin: cells chemotaxing towards cAMP from a pipette.**
- **The tip of the pipette is moved as indicated.**
- **Images were captured every 18 seconds.**
  - **From K. Barisic, M. Ecke, C. Heizer, M. Maniak, M. Westphal, R. Albrecht, G. Gerisch, Max-Planck-Institut fur Biochemie, Martinsried, Germany.**

Visualizing the activity of signal transducers in Dicty cells

- **Can look at changes in subcellular distribution of key regulators of chemotaxis**

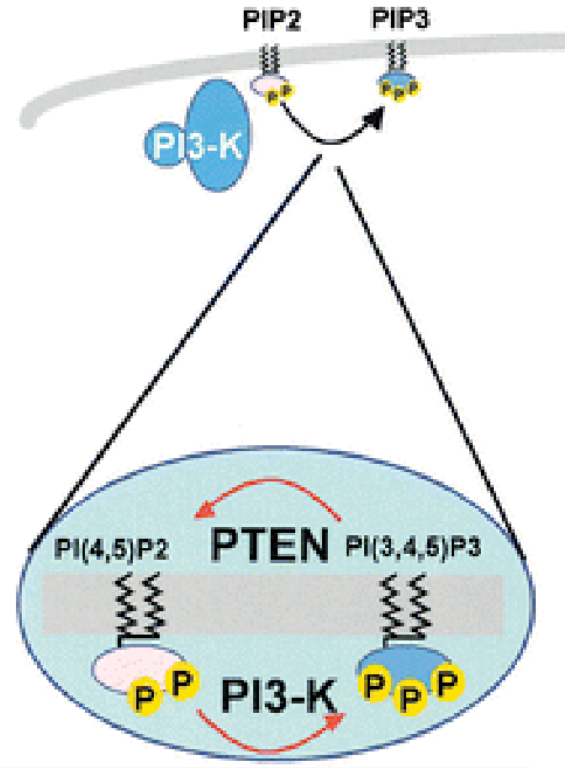
Role of phospholipid signaling in Dictyostelium chemotaxis

- **Phosphorylation of membrane lipids can serve as a localized signal to activate downstream signaling proteins**
  - **For example: Phosphorylation of PIP2 can activate PH domain containing proteins like the kinase Akt**



Two regulators of phospholipid signaling are essential for chemotaxis

- PI3-Kinase: lipid kinase
- PTEN: lipid phosphatase
- PI3-K and PTEN antagonize one another
- Both are important for robust chemotaxis

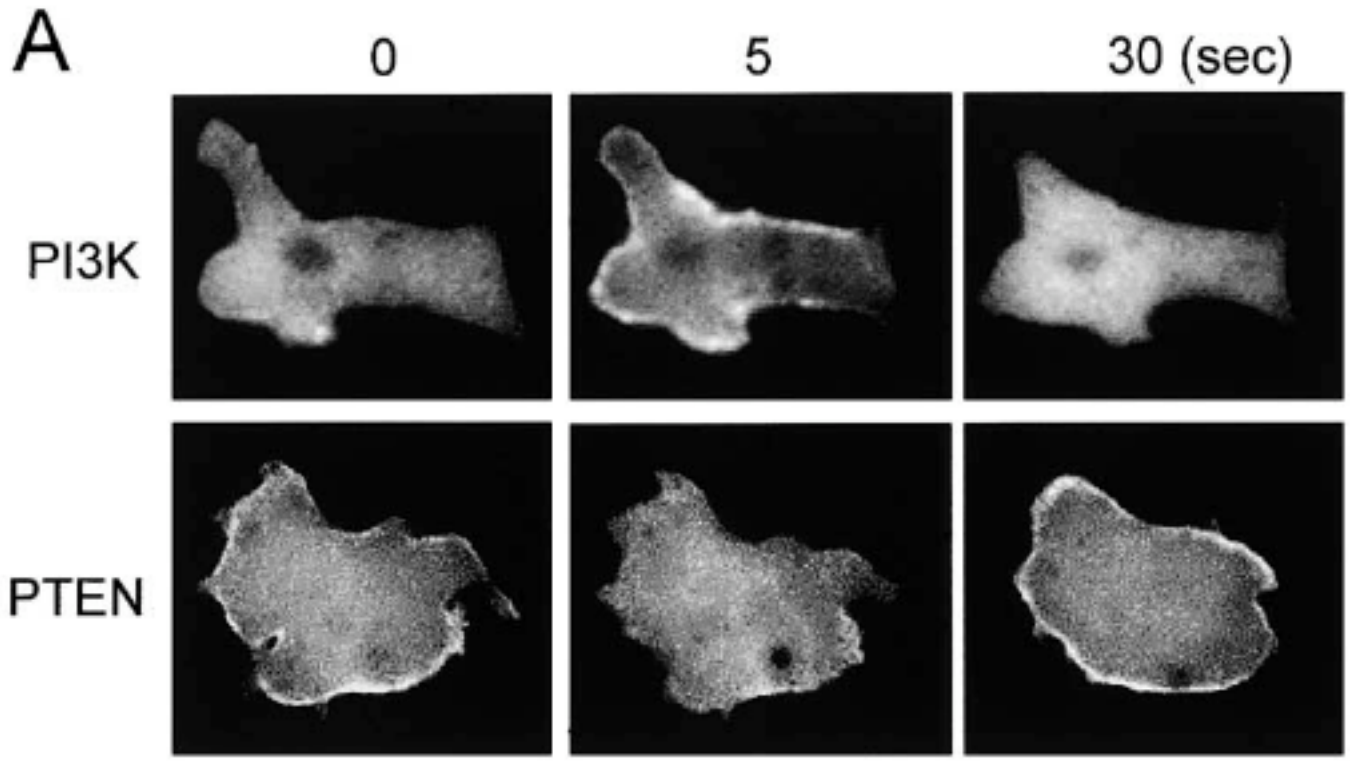


Visualizing the dynamics of PIP3 production of PIP3 in Dictyostelium

http://dictybase.org/tutorial/VIDEO1.AVI

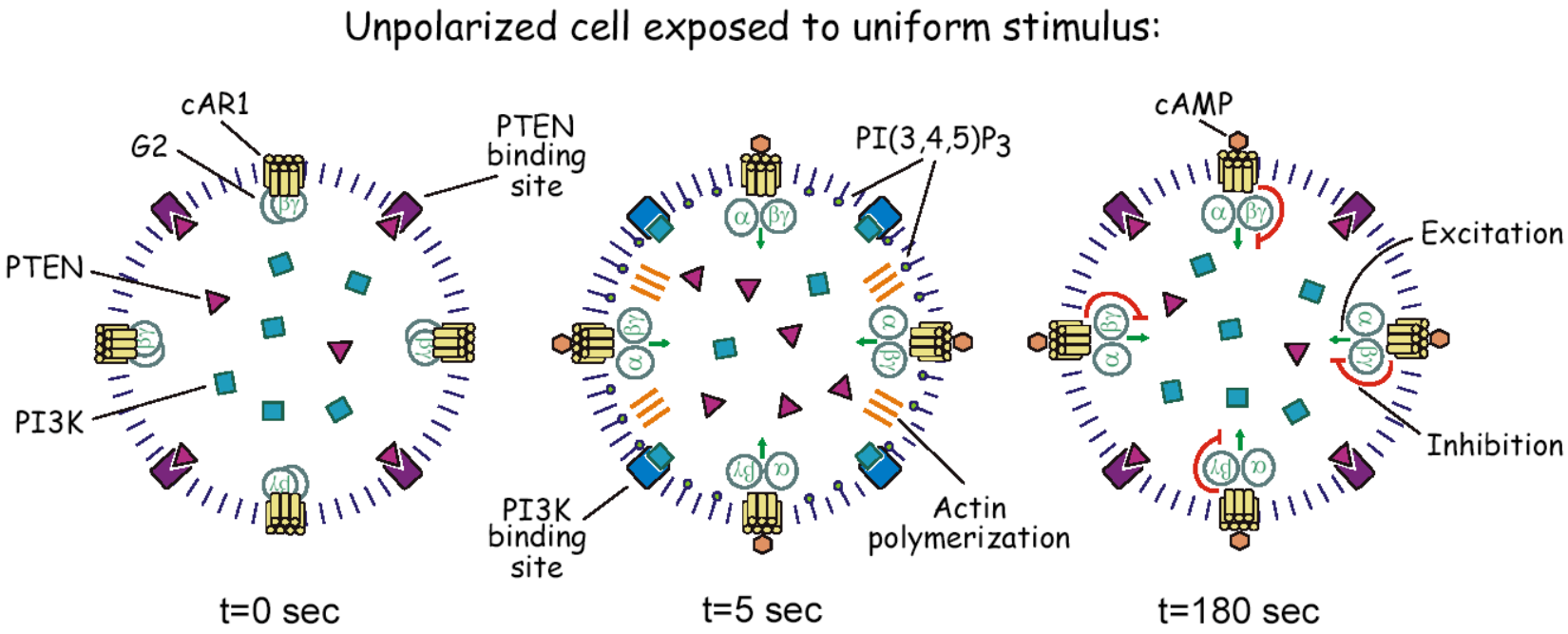
- **Translocation from to the plasma membrane of GFP-tagged PH domain in response to a uniform increase in chemoattractant.**
- **Monitors production of PIP3 by PI3-kinase (lipid kinase)**
- **See transient increase in PIP3 at membrane**
- **Frames were taken every 2 seconds.**

PI3-K and PTEN alternate at the membrane



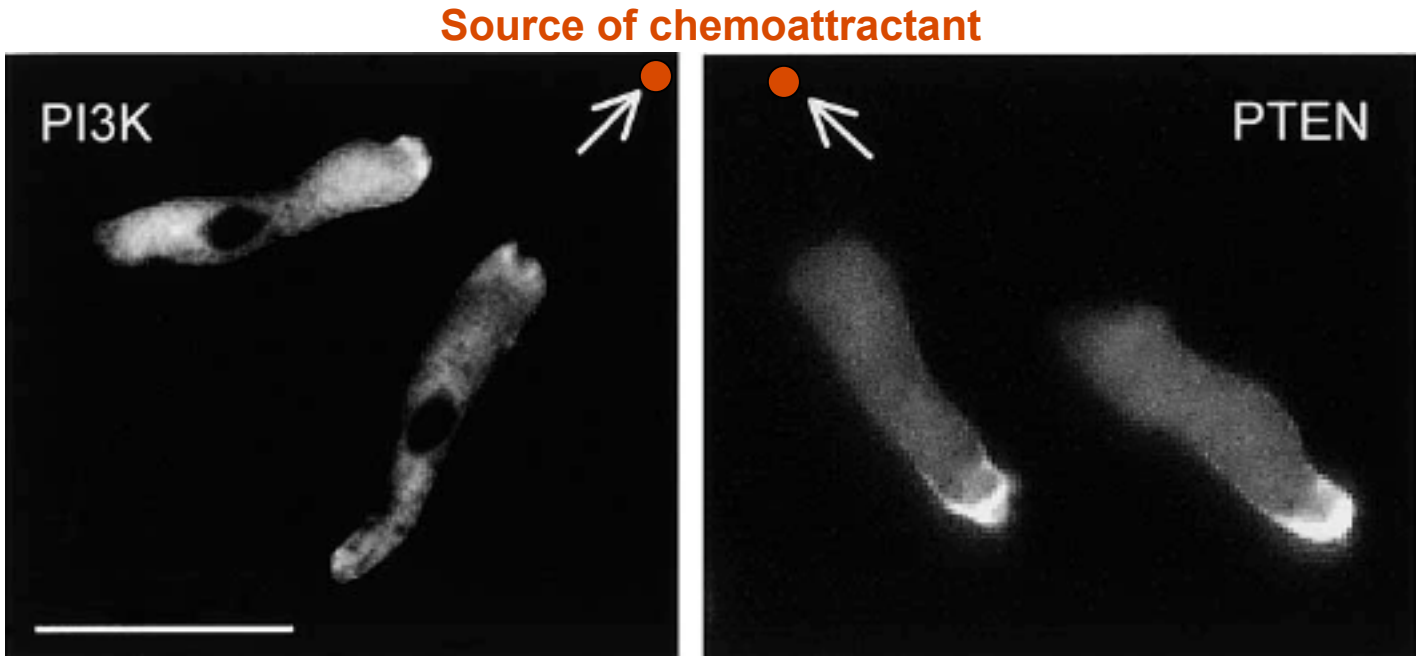
Model of PIP3 production

- **Alternating activation/inhibition of PIP3 production**

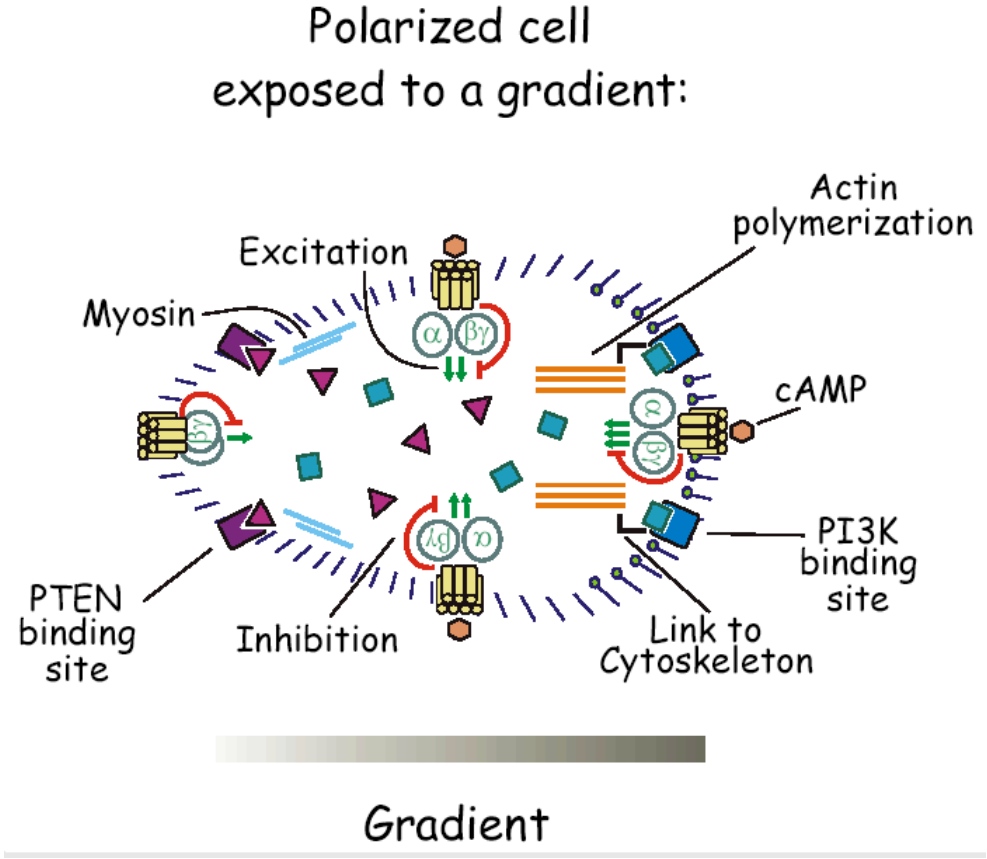


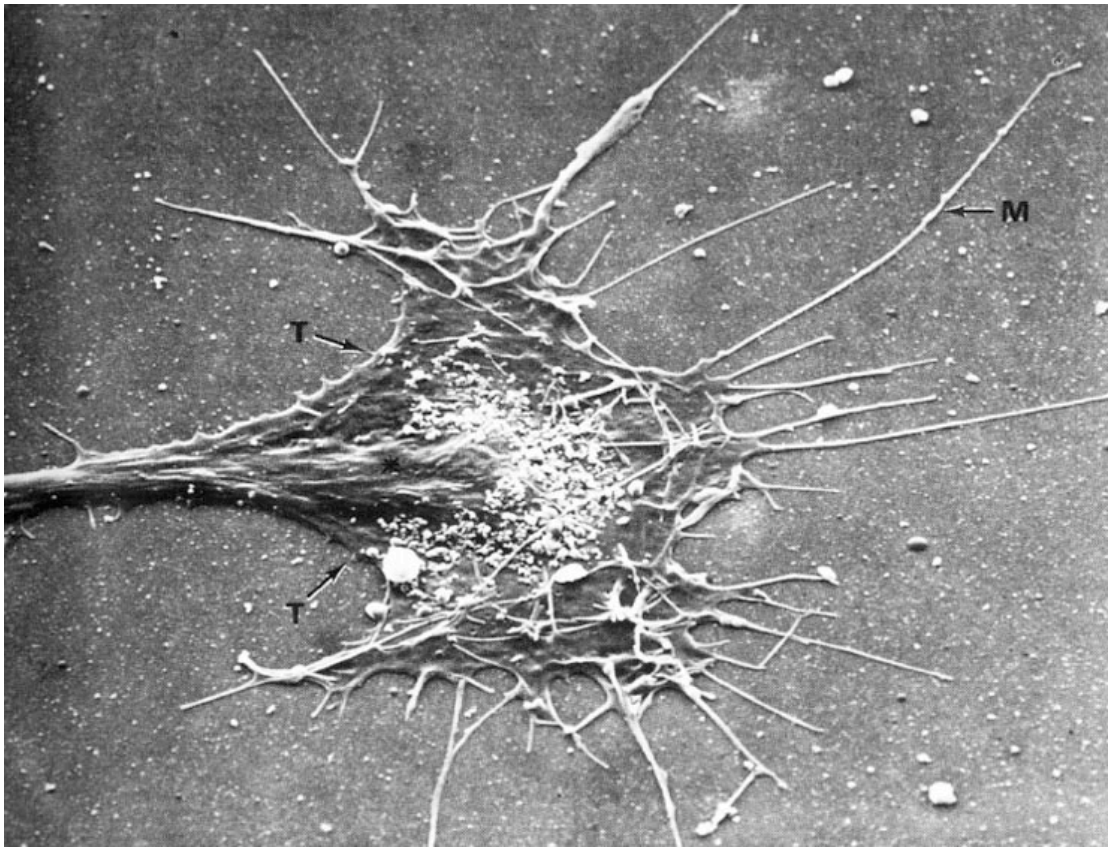
Visualizing the enzymes responsible for controlling PIP3

- See increase in PI3K at leading edge
- See increase in PTEN at trailing edge



Current models of response of Dictyostelium cells to chemoattractant

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