Axon Guidance

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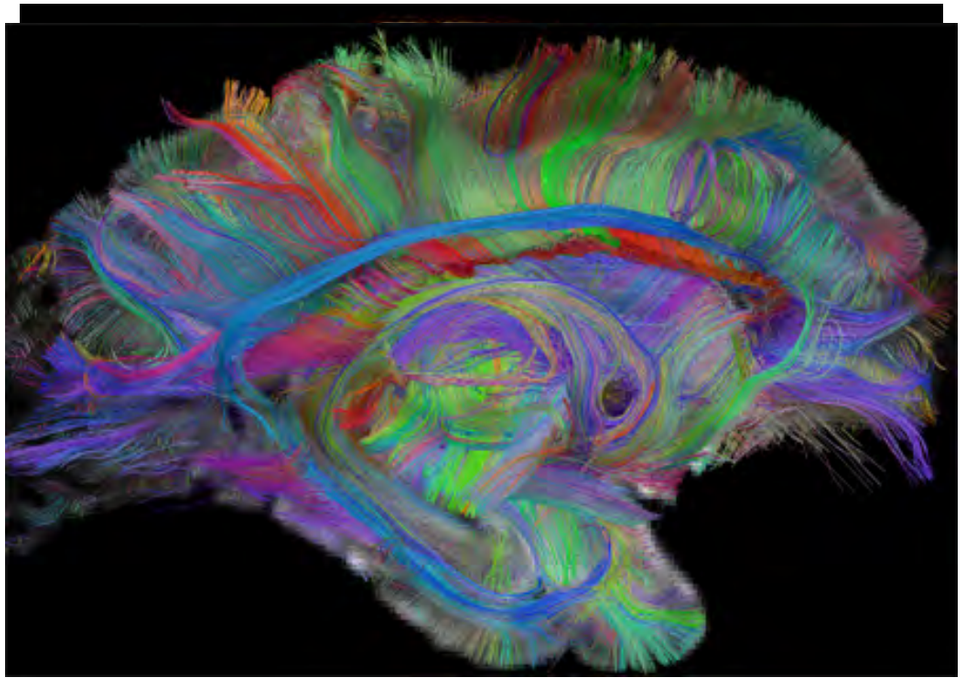
Chapter 5; **Development of the Nervous System,** Sanes et al.

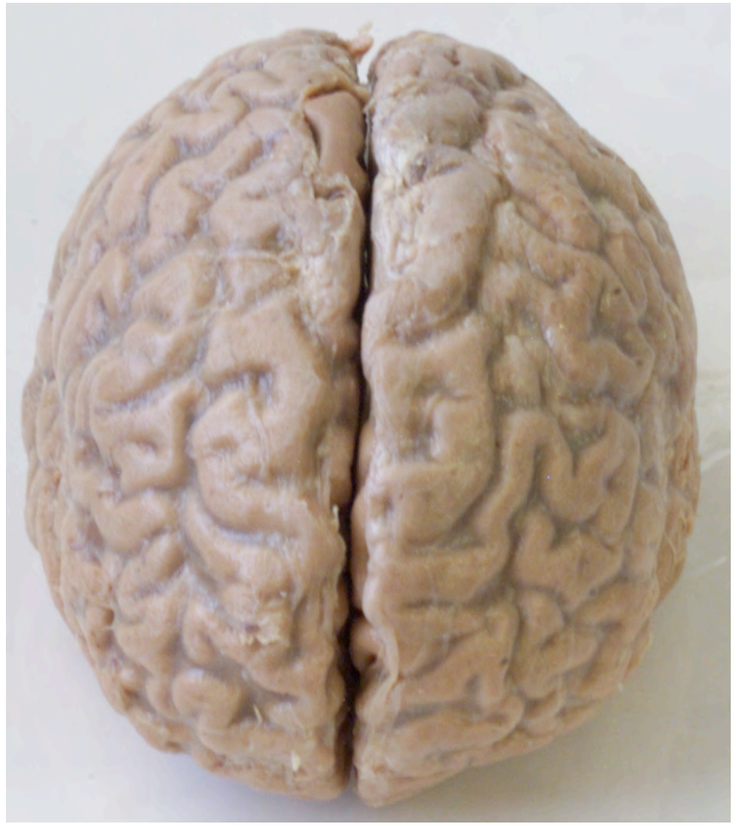
Axon guidance concepts

- Growth cone is the navigator for axonal pathfinding.
- Growth cones express surface receptors for guidance cues, and the receptors trigger regulation of growth cone motility to produce turns and navigation.
- Local guidance cues change as an axon moves along its path.
- Adhesive cues mark pathways for axon elongation.
- Locally produced guidance molecules act as attractants and/or repellents of axons.
- A single cue can be attractive or repellent, depending on the growth cone response.

- In developing limbs targeting of axons to dorsal vs ventral targets depends on neuronal expression of receptors and spatial expression of guidance cues.
- At the midline, spatial and temporal regulation of expression of guidance cue receptors regulates guidance.

- Growth cone navigation involves locally regulating actin protrusion, adhesion and advance of microtubules.
- Ligand-receptor binding triggers cytoplasmic signaling that regulates the dynamics of growth cone actin and cell-substrate adhesion.
- Several intracellular signaling systems contribute to modulating guidance cue regulation of growth cone motility.

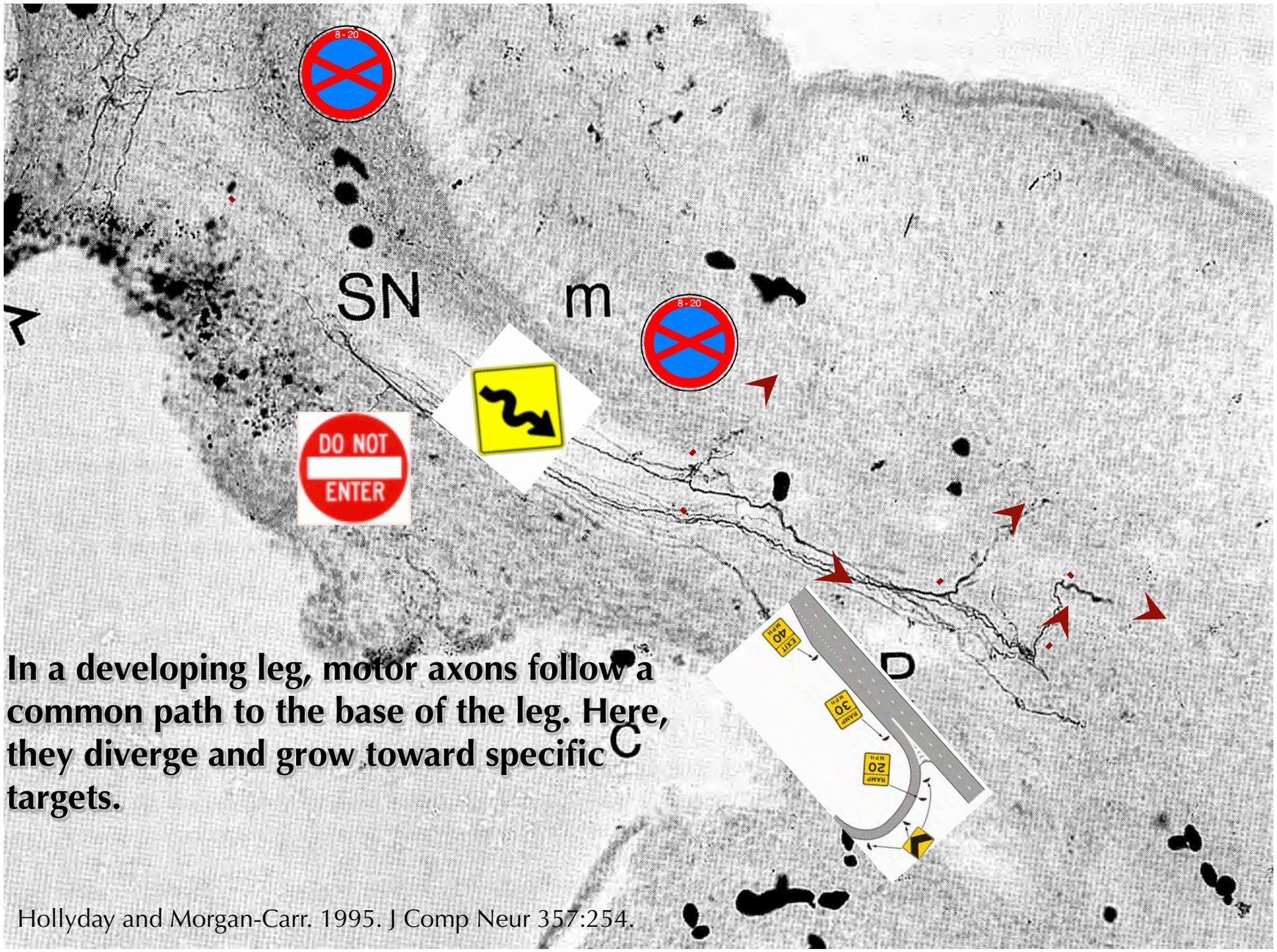




- 100 billion neurons
- 100 trillion synapses

How do neural circuits develop?
How do axons get to their targets?

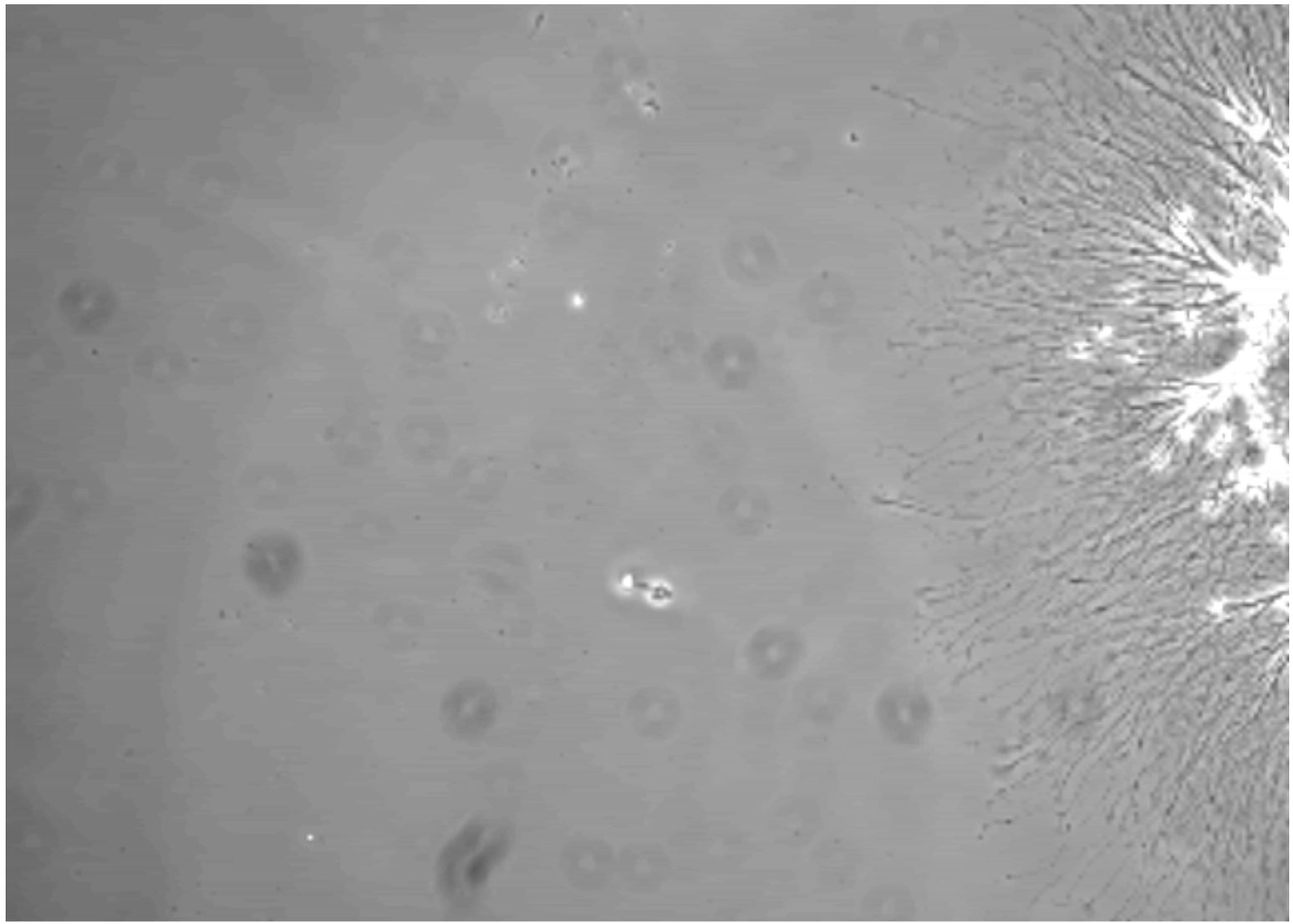
The migration of an axonal growth cone from a nerve cell body to a synaptic target is like a road trip.



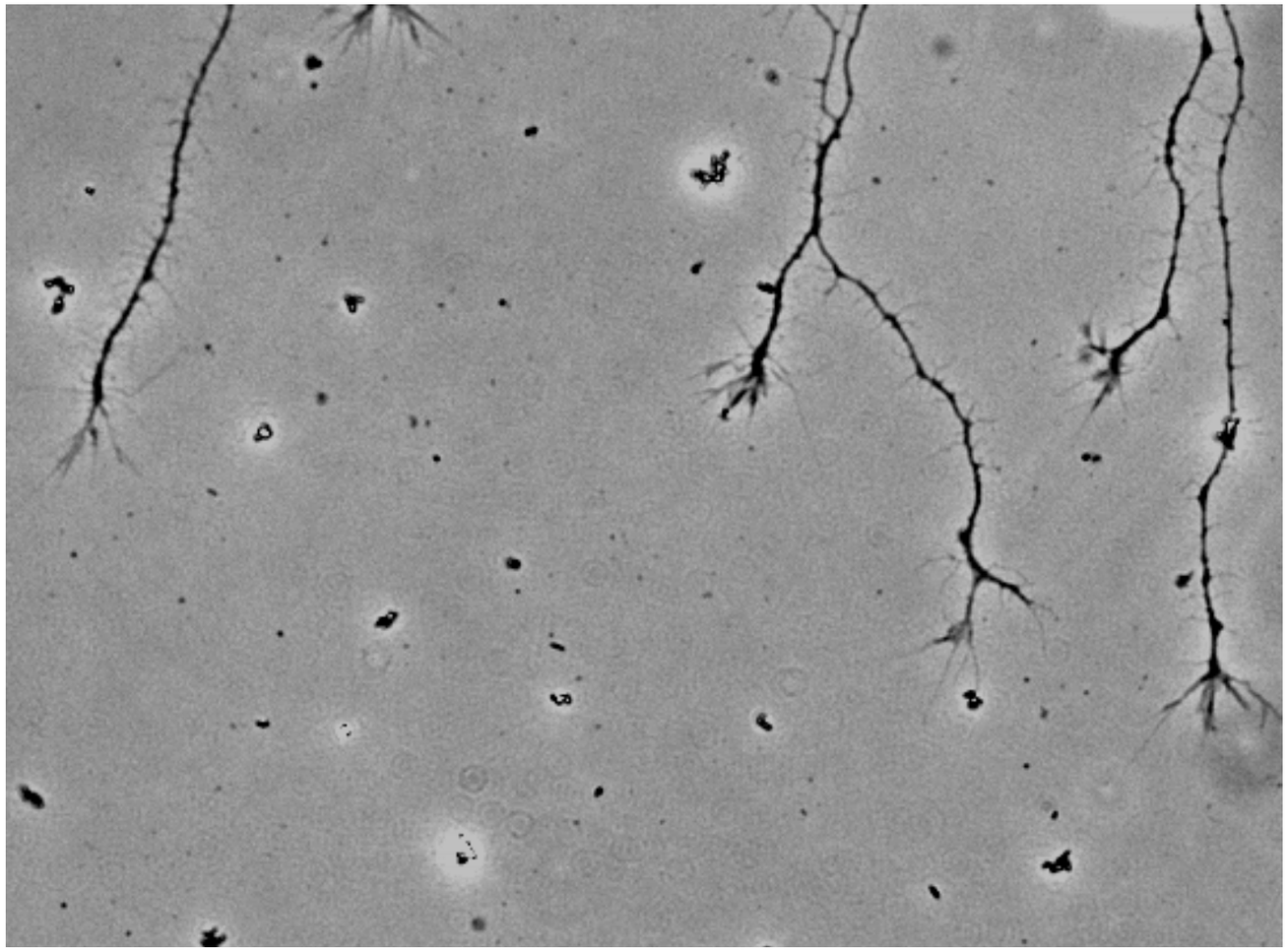
In a developing leg, motor axons follow a common path to the base of the leg. Here, they diverge and grow toward specific targets.

Hollyday and Morgan-Carr. 1995. J Comp Neur 357:254.

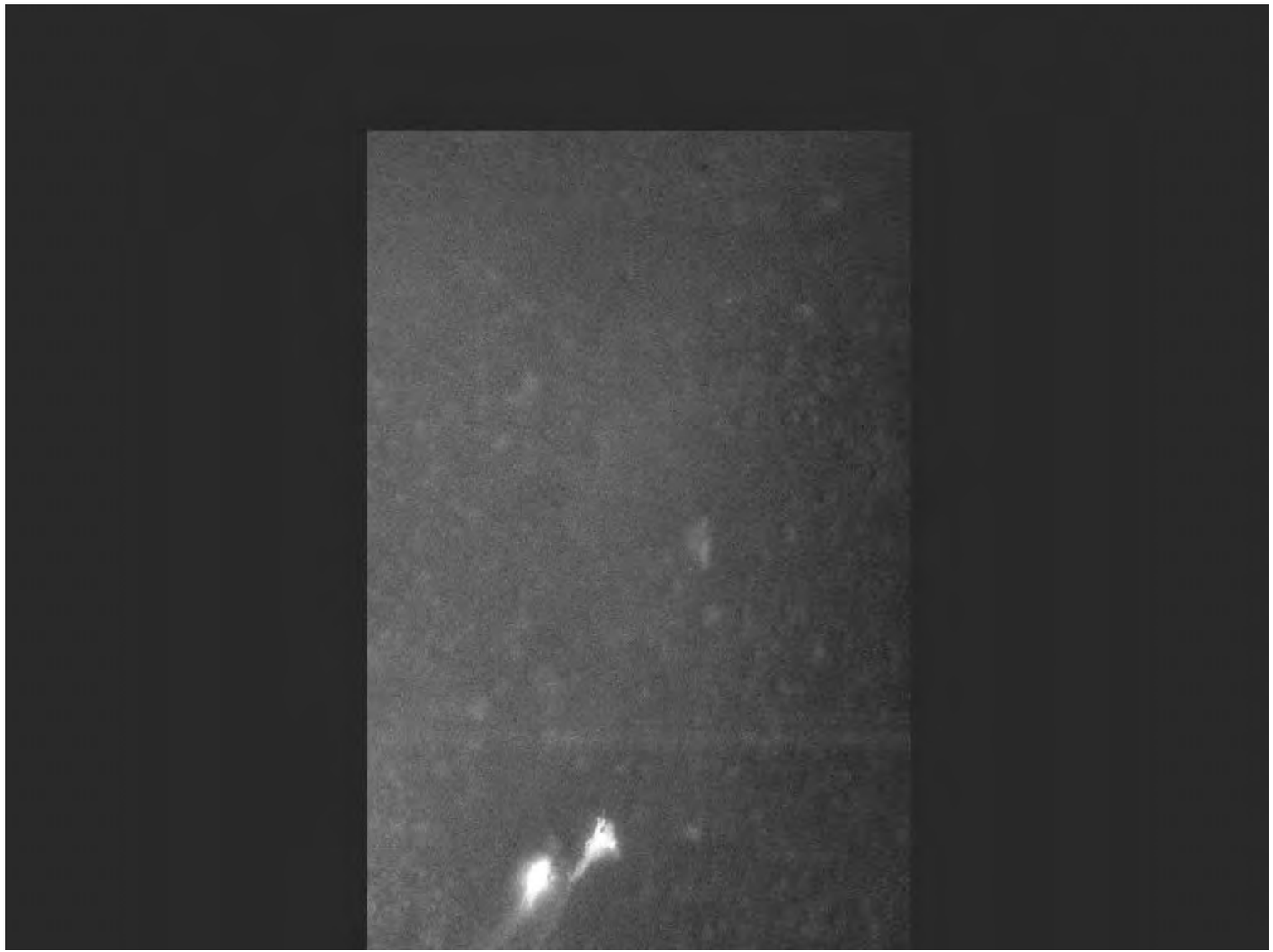
•Axon elongation involves the advance and assembly of microtubules and the addition of membrane to expand the plasma membrane.

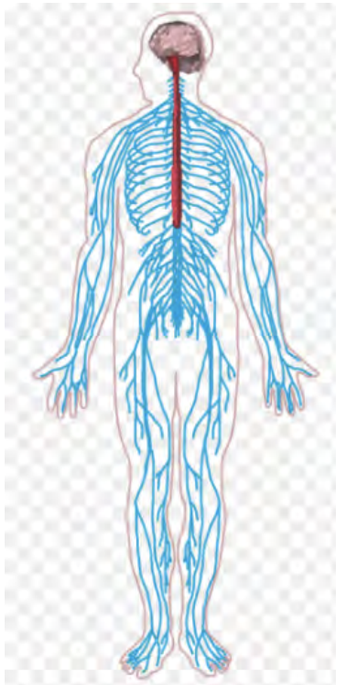


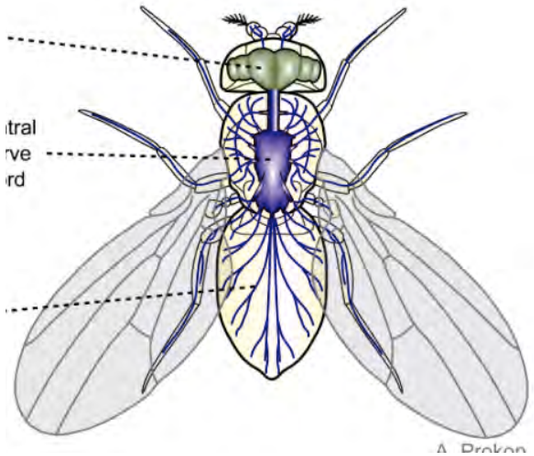
- A Growing axon is tipped by a motile growth cone. The growth cone leads the growing axon to its target.
- The dynamic assembly and turnover of actin filaments at the growth cone protrudes its leading margin and promotes exploration for guidance cues.
- Surface receptors on growth cones detect guidance cues that regulate growth cone motility to produce guidance.



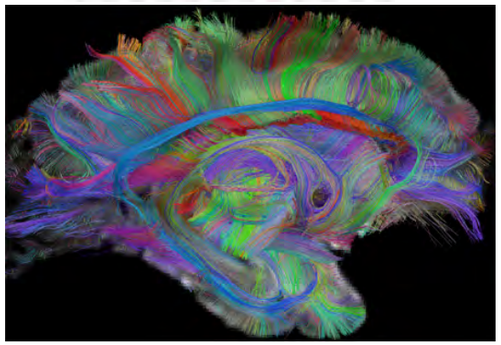
Growth cones *in vivo* closely resemble growth cones *in vitro*







3-4 mm
2.5×10^5 neurons
5×10^6 synapses
12 days

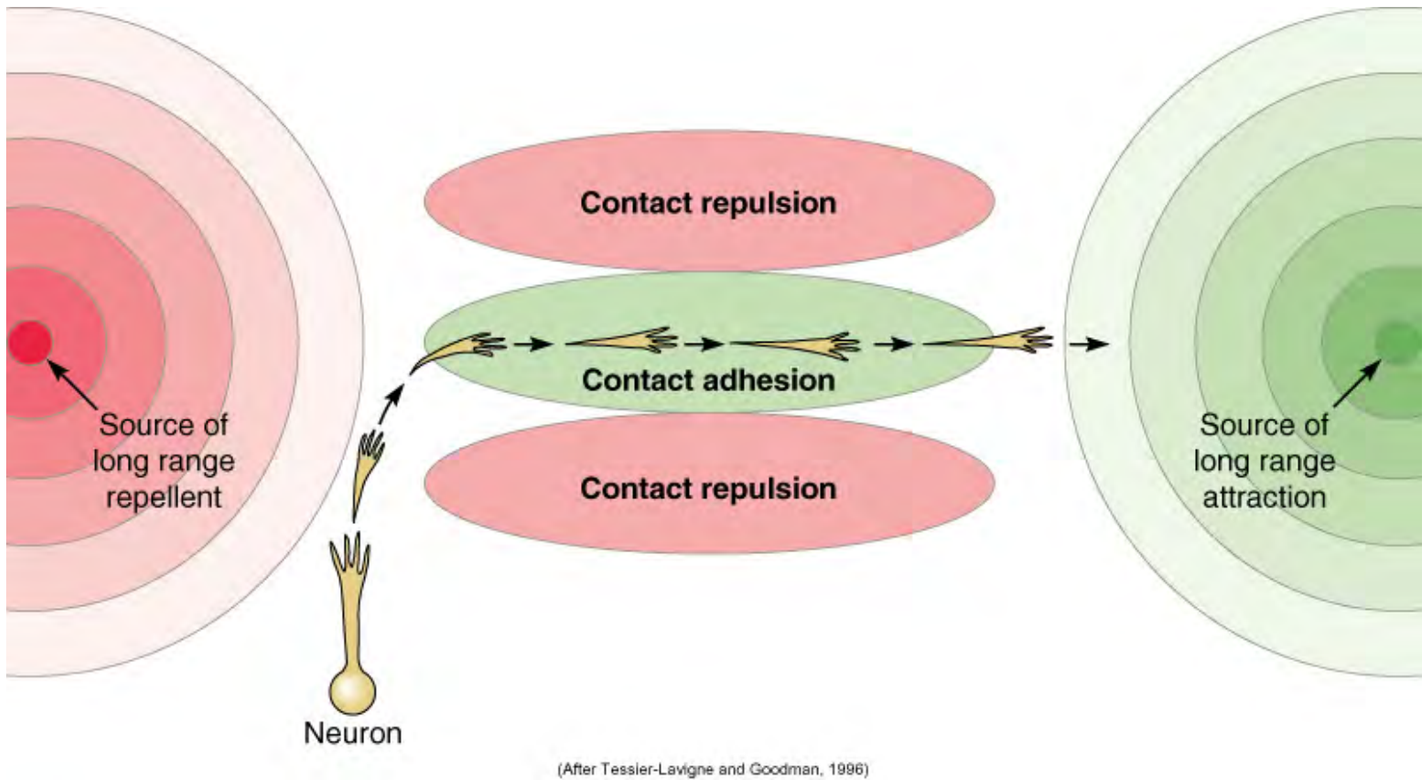


10^{11} neurons
10^{14} synapses

1 mm
3×10^2 neurons
7×10^3 synapses
3.5 days

15+Guidance mechanisms and guidance molecules are conserved across millions of years

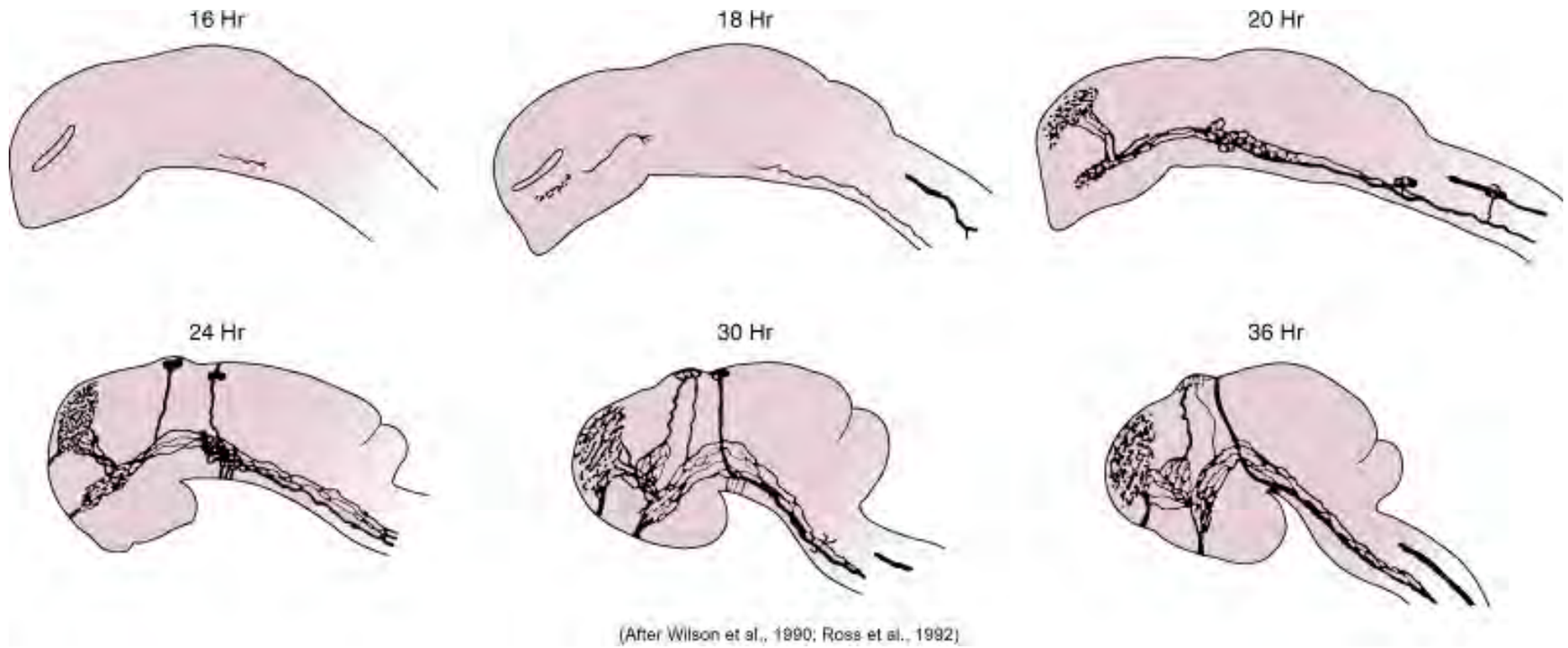
- Growth cone navigation involves interactions with multiple environmental guidance cues.
- Each segment of the navigation pathway involves interactions with a different set of guidance cues.



(After Tessier-Lavigne and Goodman, 1996)

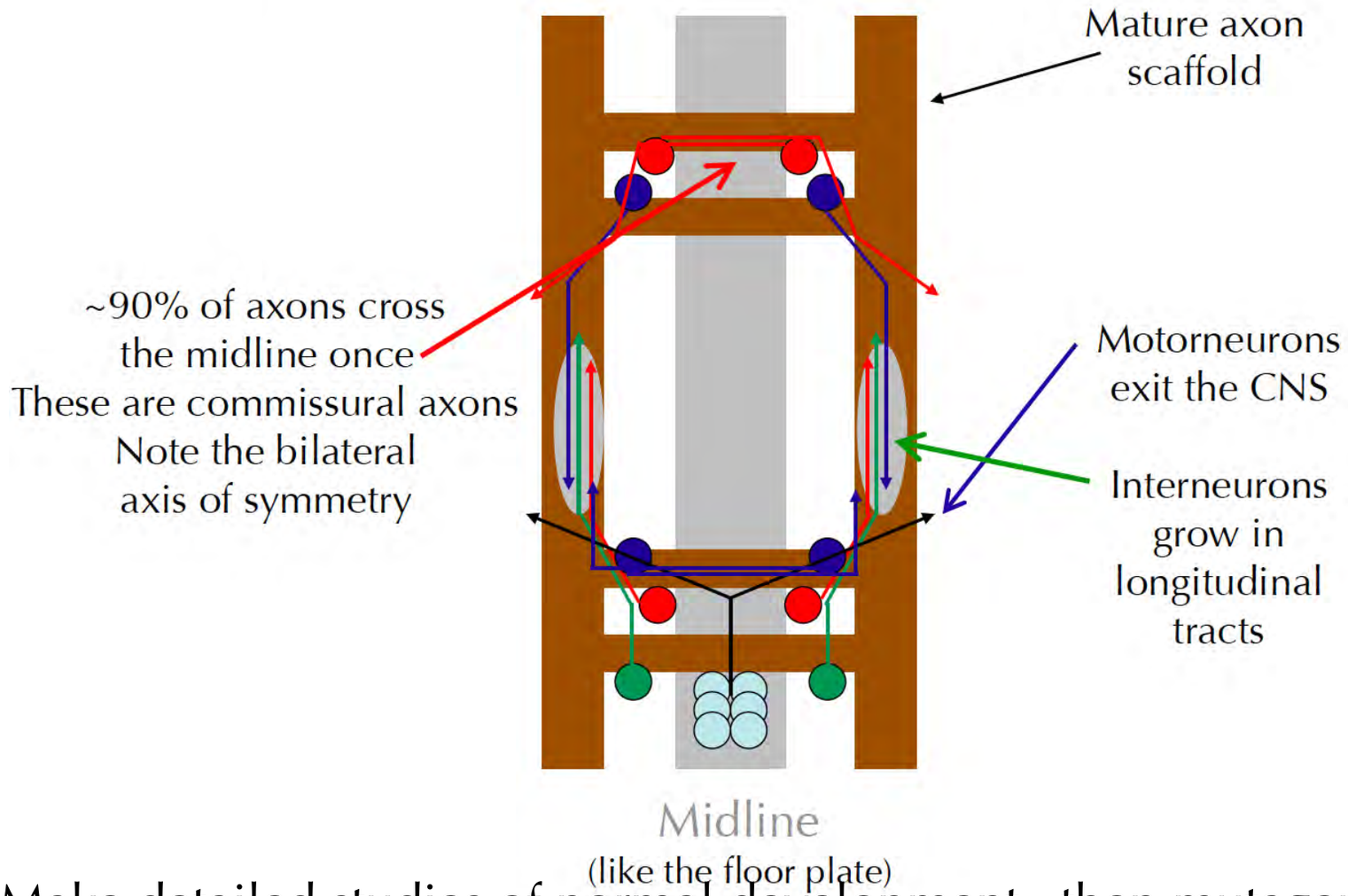
•In a zebrafish embryo axonal pathways develops through a regular pattern of adding new axons.

•Previously formed axon bundles become pathways for later following axons.



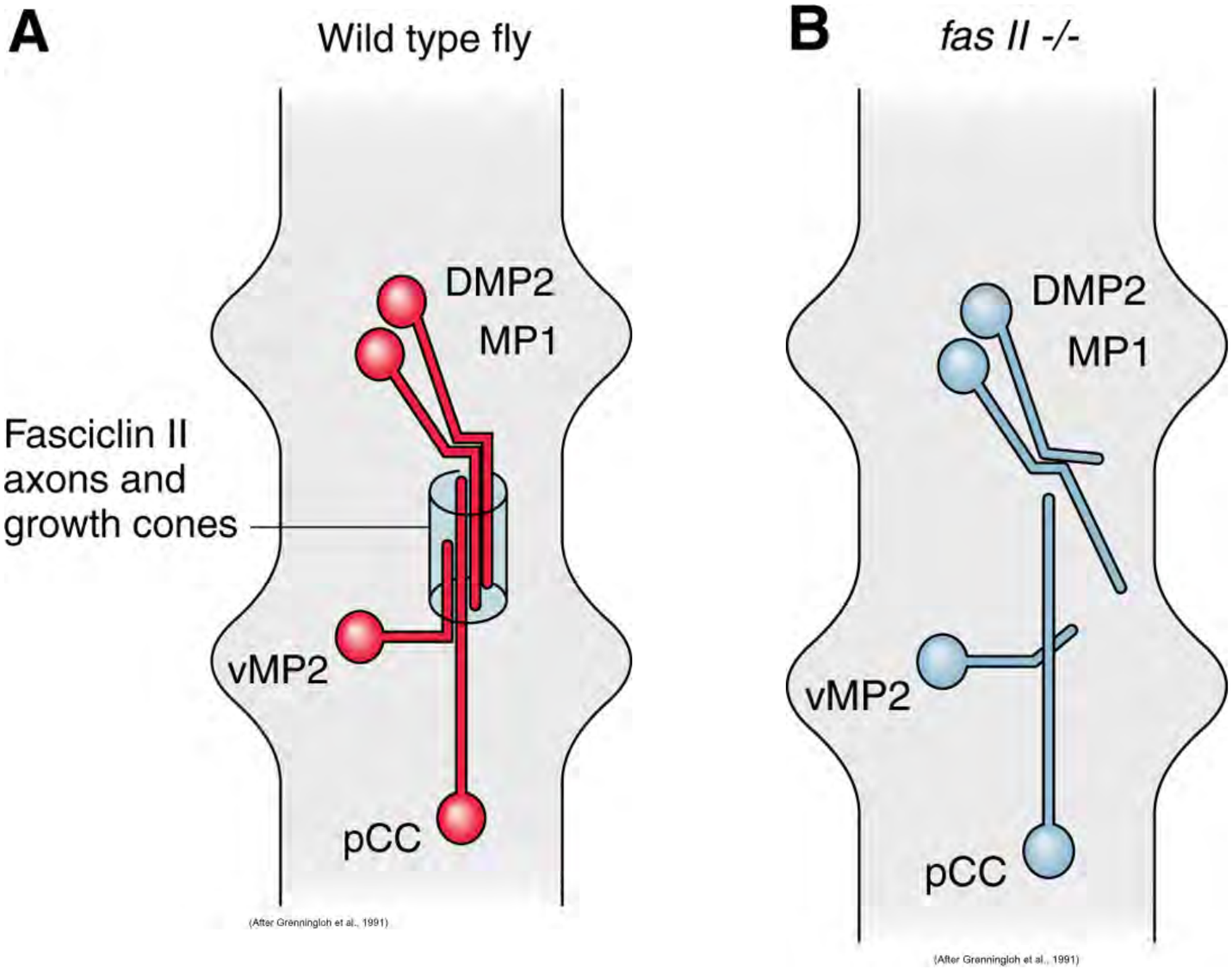
(After Wilson et al., 1990; Ross et al., 1992)

Drosophila Embryonic Central Nervous System



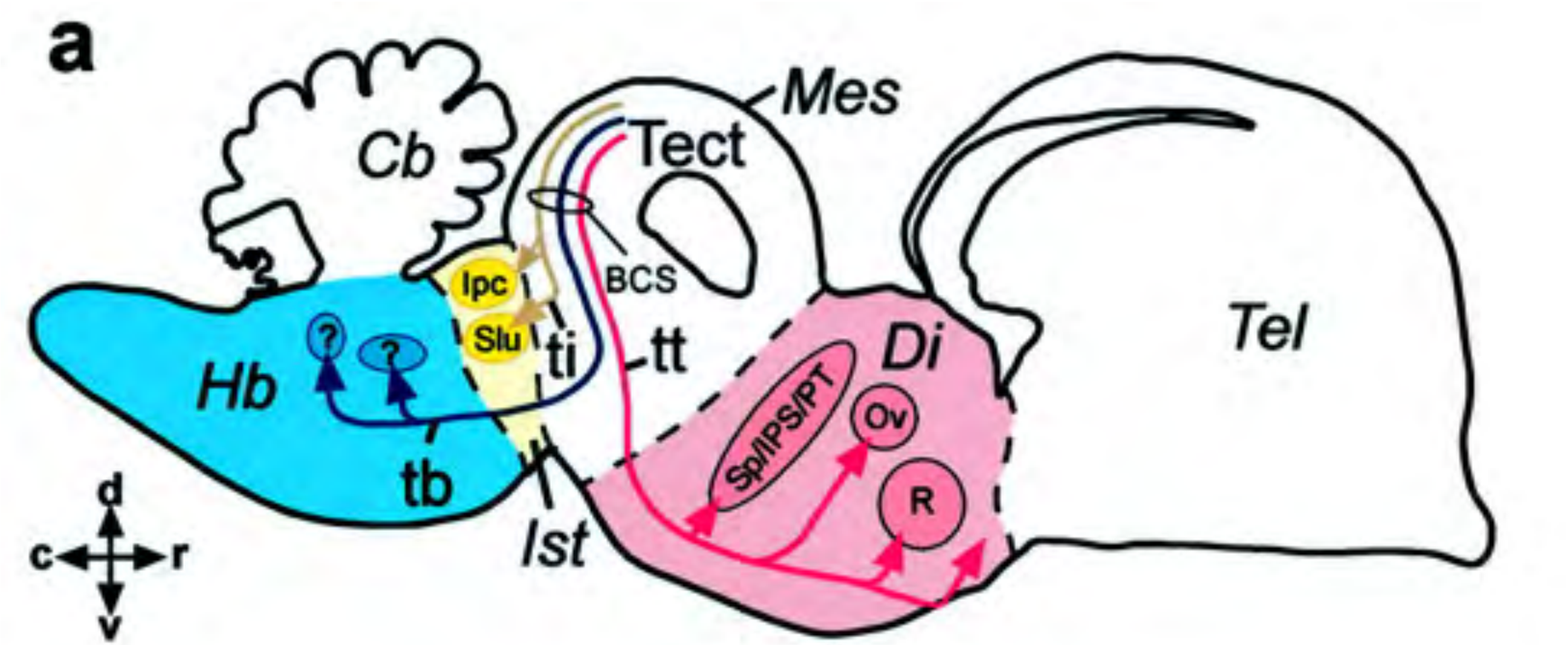
Make detailed studies of normal development– then mutagenize flies and look for abnormal neural development in their offspring.

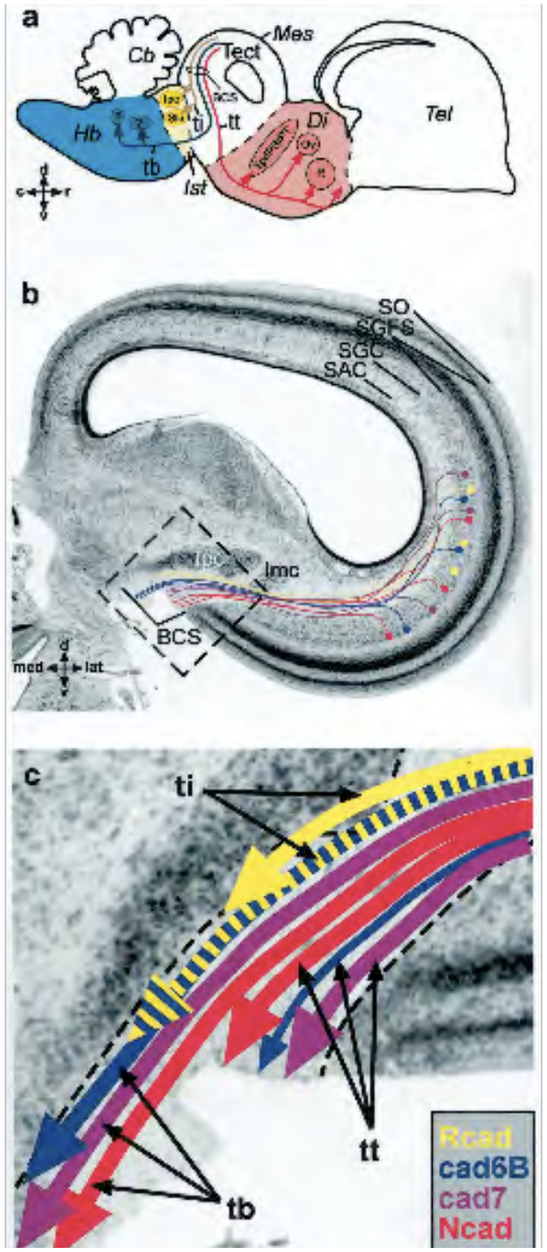
Growth cones follow previously formed axonal fascicles marked by specific adhesion molecules, in this case, fasciclin II.



Adhesion molecules mark specific pathways in vertebrates

Axonal projections from optic tectum go to three CNS targets; diencephalon (Di), isthmus (Ist), hindbrain (Hb)





Axonal projections from optic tectum go to three CNS targets; diencephalon (tt), isthmus (ti), hindbrain (tb)

Axons projecting to these three targets express different combinations of cadherins:

tt (N-cadherin, cadherin6B, cadherin 7)
Tb (N-cadherin, cadherin6B, cadherin 7)
Ti (cadherin6B, R-Cadherin)

Adhesion molecules regulate sorting of axons to specific targets

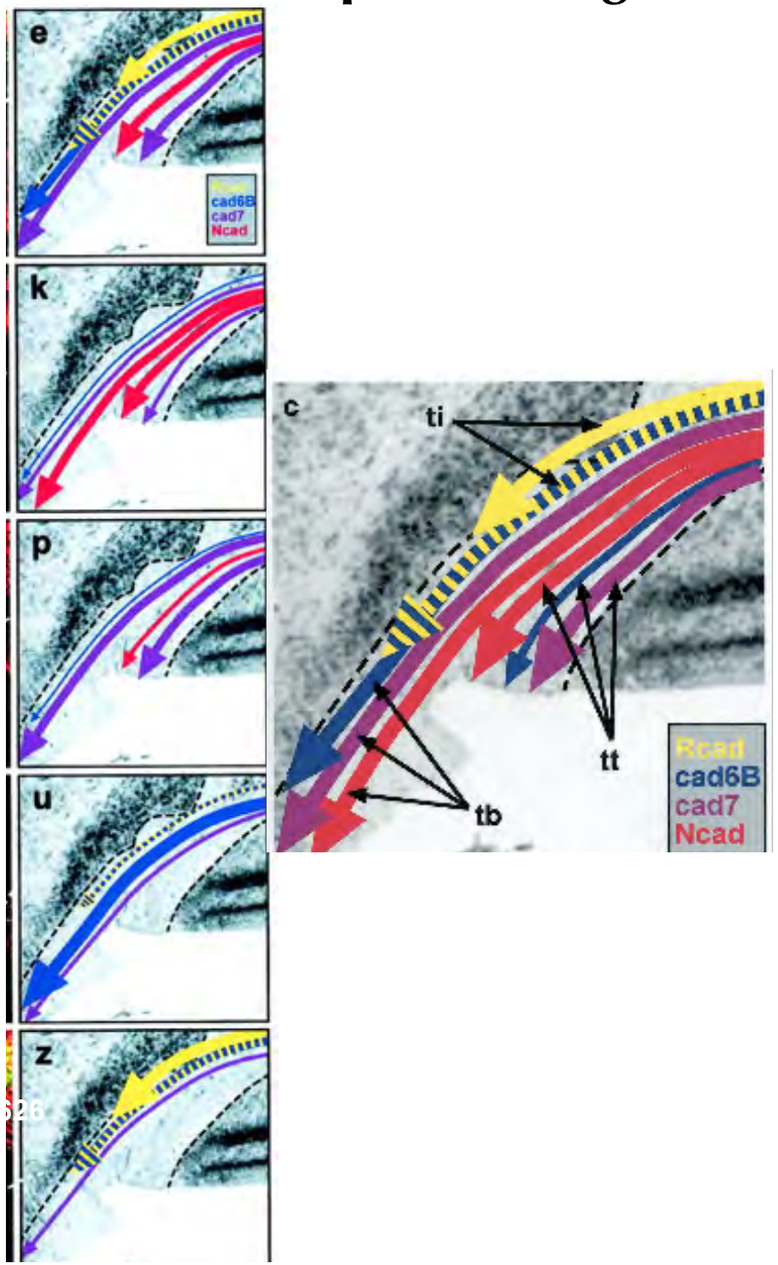
GFP expressing axons
Normal distribution

Express extra Ncad on axons
Most go to tt and tb

Express extra Cad7 on axons
Go with other cad7 expressing axons

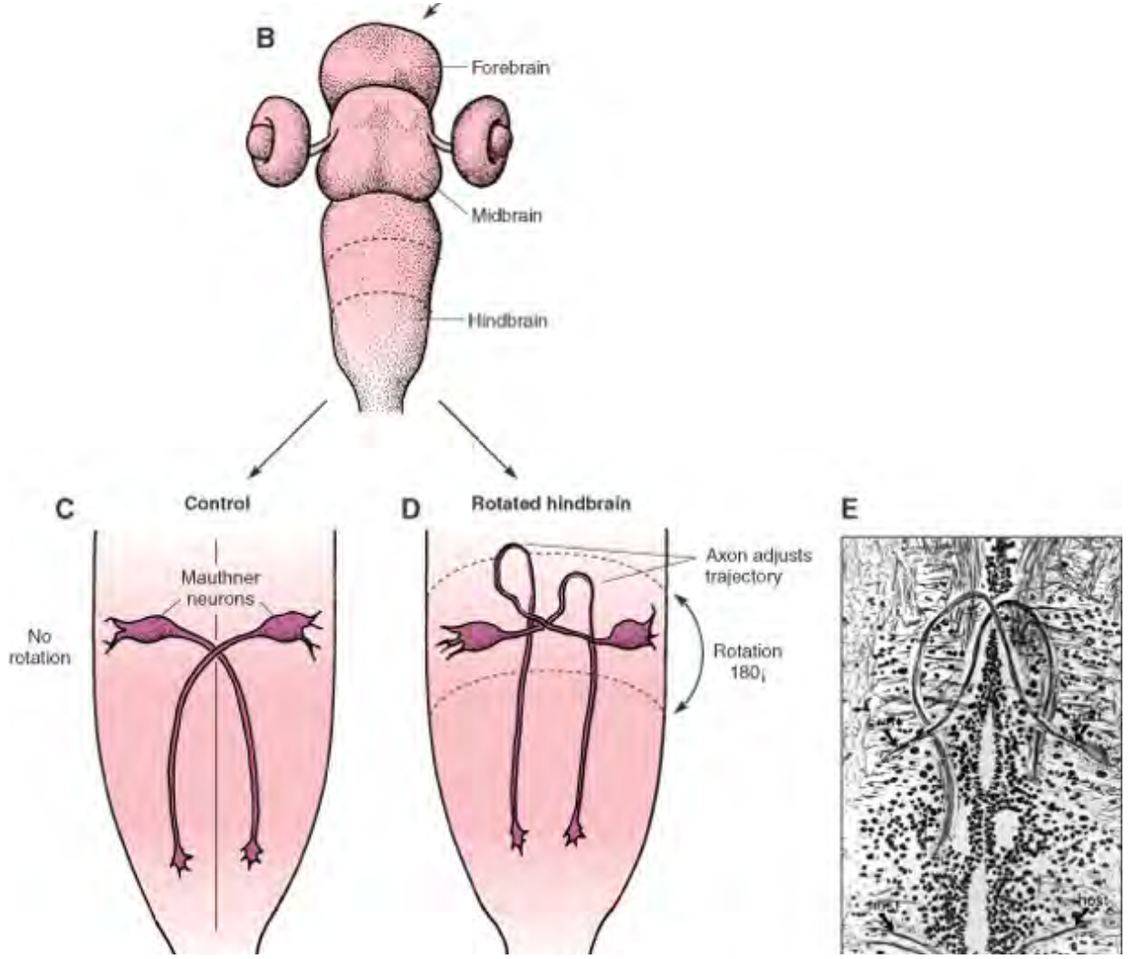
Express extra Cad6B on axons
Most go to tb with other cad6B axons

Express extra Rcad on axons
Most go to ti



Axons are guided by cues in their local environment

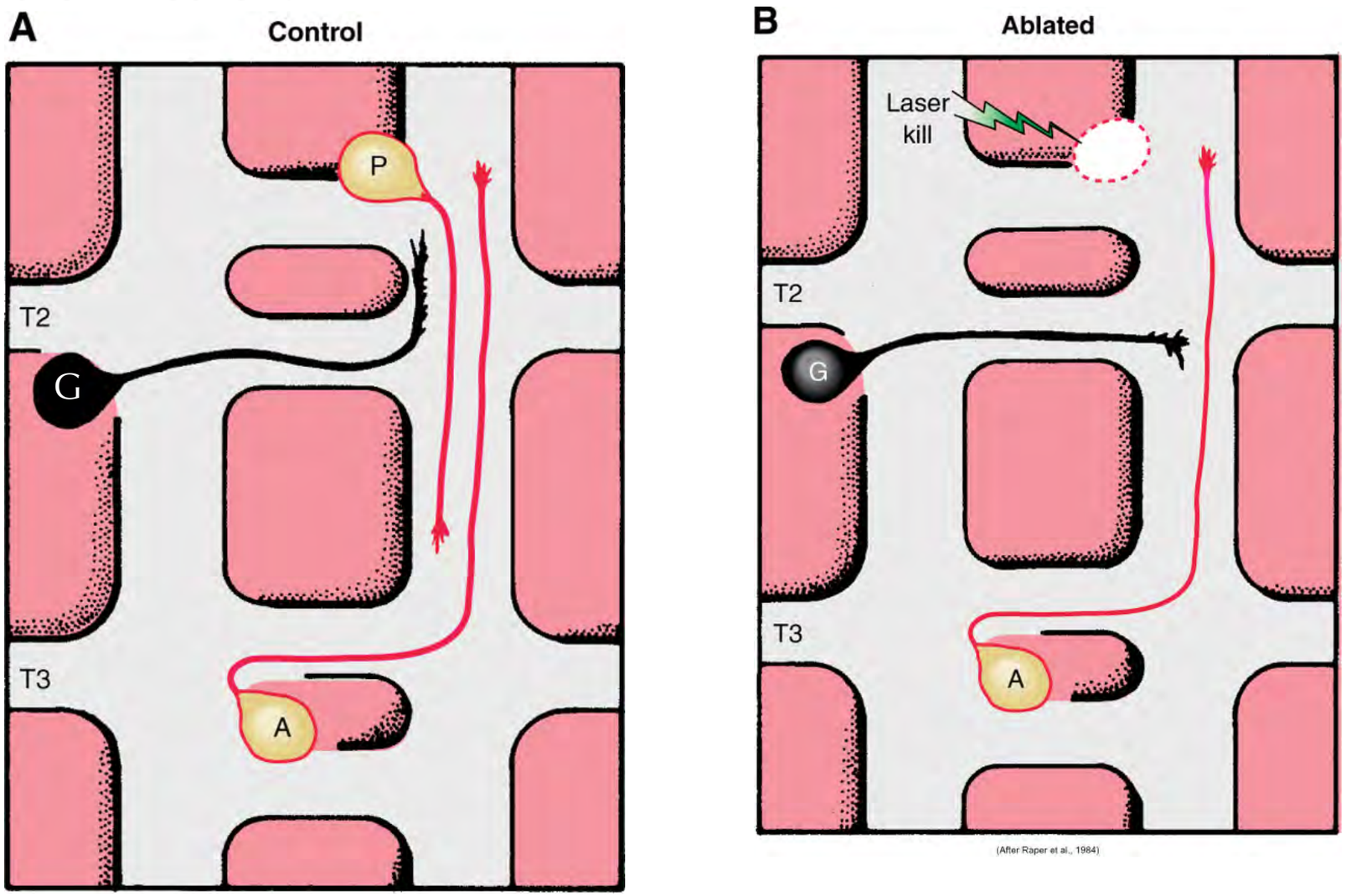
- Axons of Mauthner cells are guided posteriorly through the hindbrain by cues in the local environment.



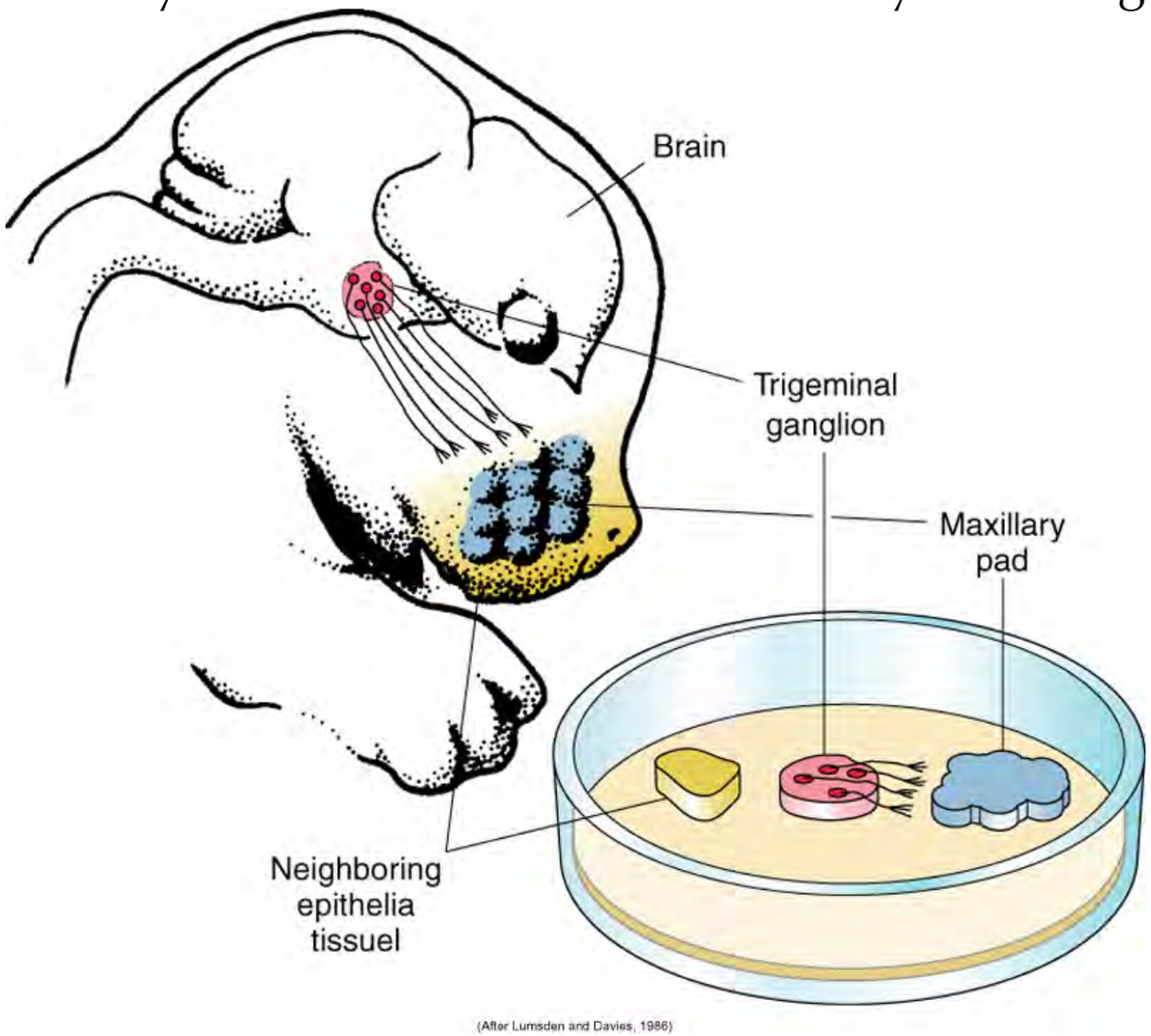
- These cues allow axons to sense direction or polarity in their environment.

Specific cell-cell interactions can determine axon pathways

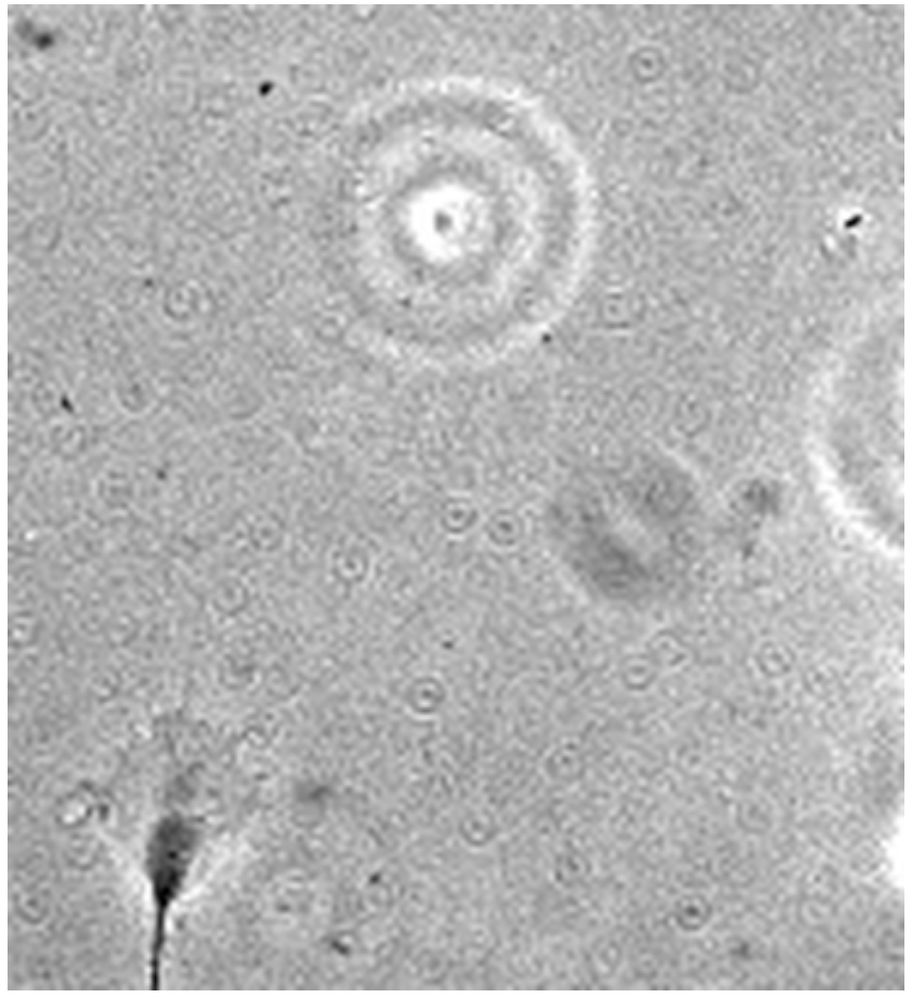
The G neuron growth cone turns rostral at the A-P fascicle. Correct turning of the G neuron growth cone requires recognition of the axon of the P neuron.



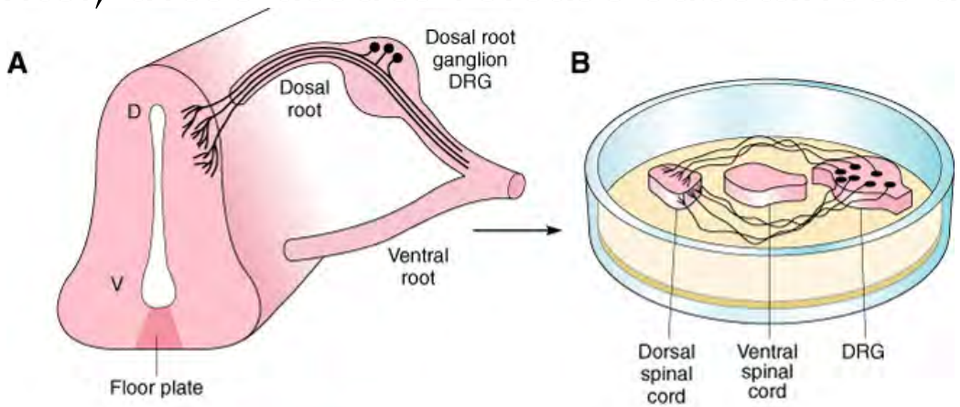
Trigeminal sensory axons grow a few mm to their target organ, the developing whisker pad. Trigeminal growth cones in tissue culture are attracted by soluble molecules released by their target.



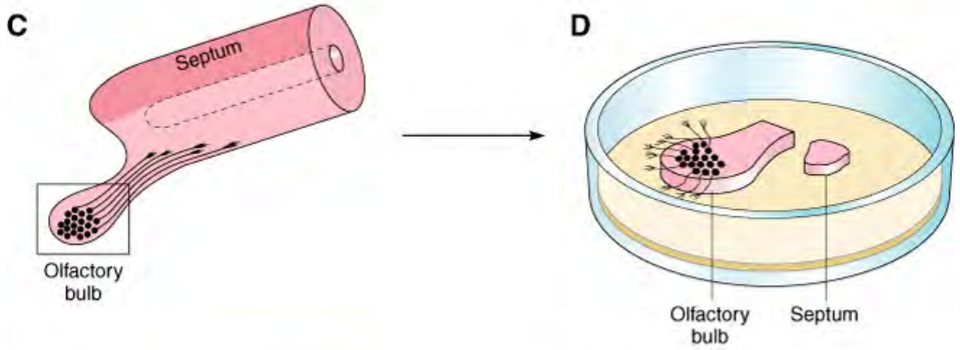
Local application of the attractant netrin induces retinal growth cone turning toward the source of netrin



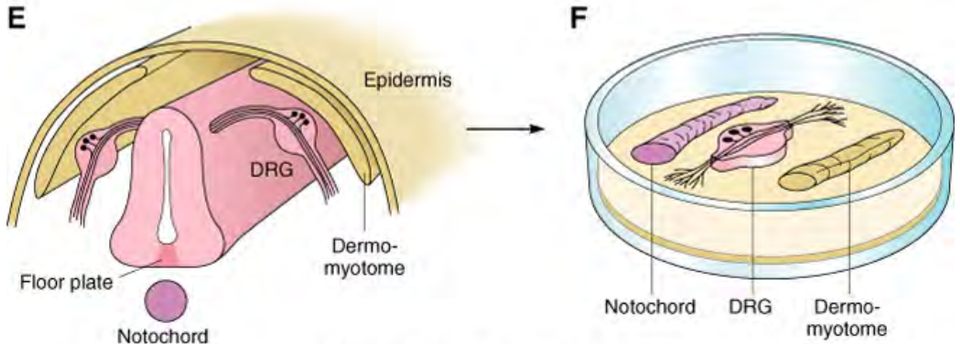
When neurons are combined with tissues that their axons do not enter, these tissues release substances that repel axons.



Central axons of DRG neurons are attracted to dorsal SC, while they are repelled by ventral SC.

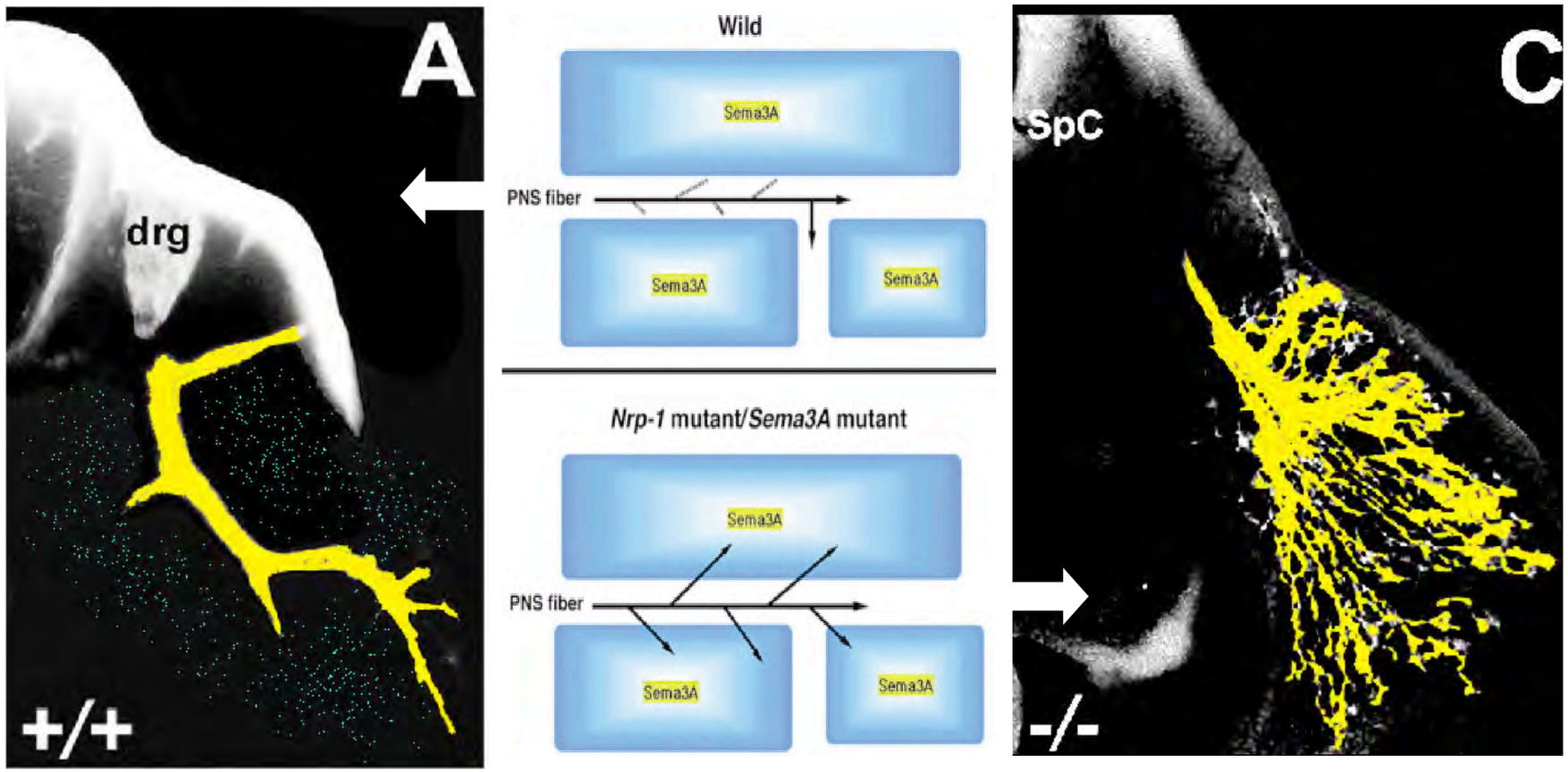


Navigation of axons away from the olfactory bulb involves repulsion by the molecules from the septum.

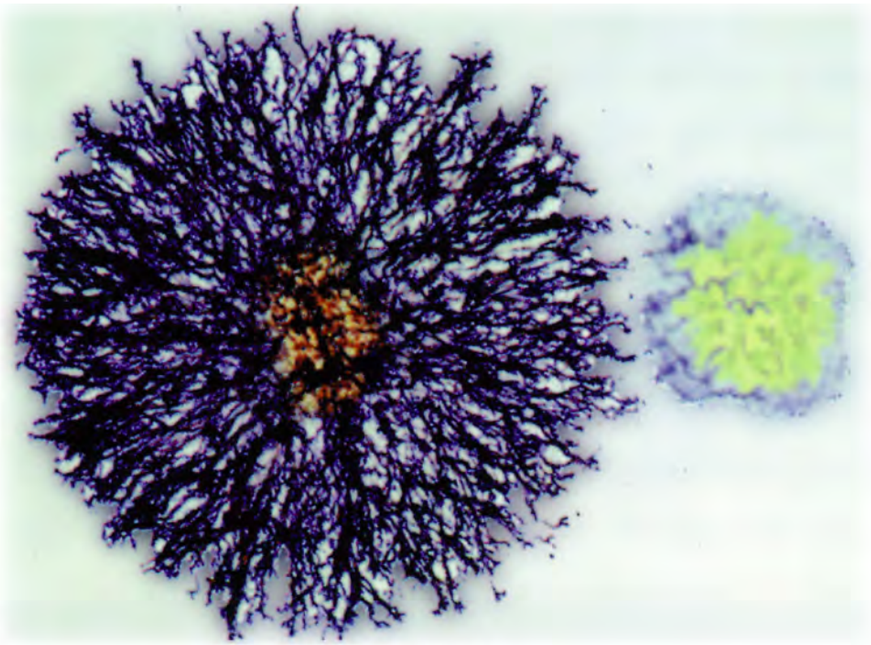


Peripheral axons of DRG neurons are channeled by repulsion from molecules released by the notochord and the dermamyotome

- **Semaphorin 3A** is present in regions that peripheral axons avoid
- When sema3A or its receptor are absent, axonal bundles break up, and axons go off course abnormally. (White and Behar, 2000)



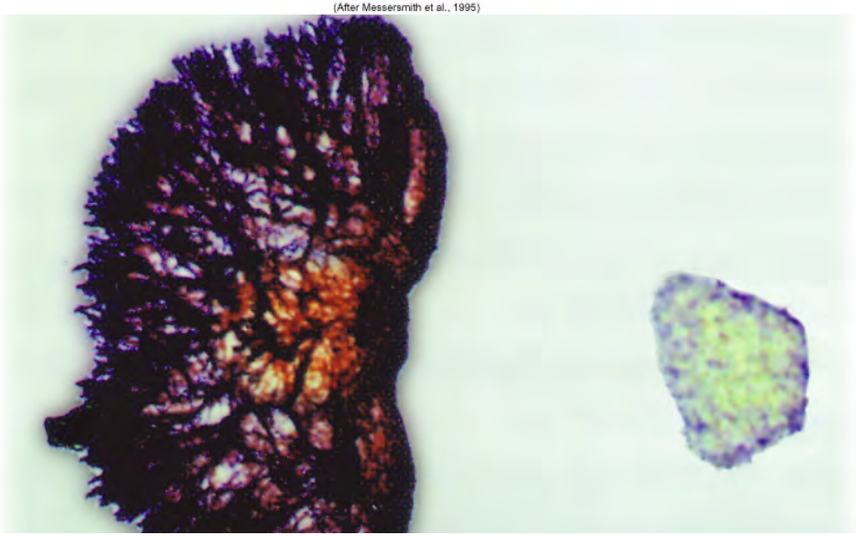
Negative cues induce axons to turn away from their source



Dorsal root ganglia
Control COS cells

B

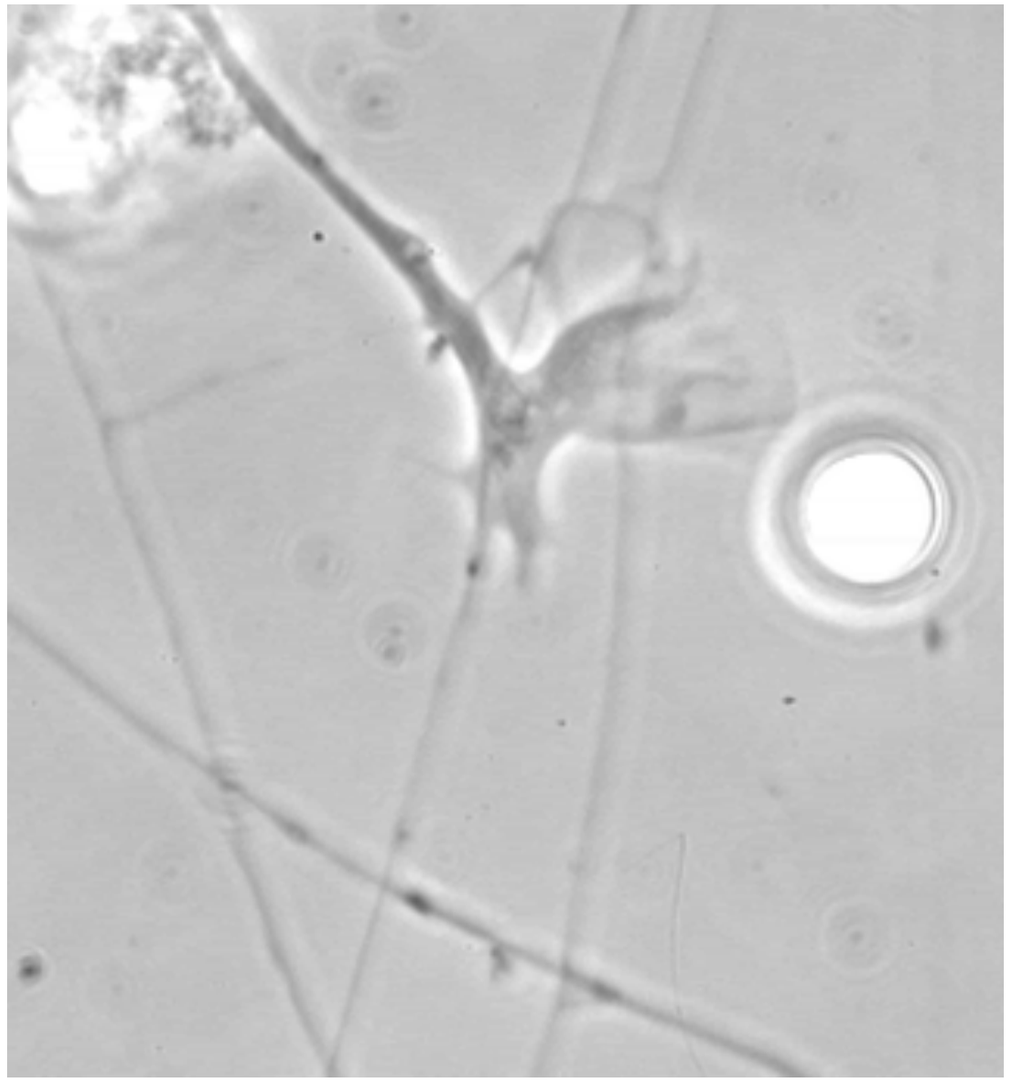
(After Messersmith et al., 1995)



Axons of DRG neurons are repelled from a gradient of Semaphorin3A released from transfected COS cells

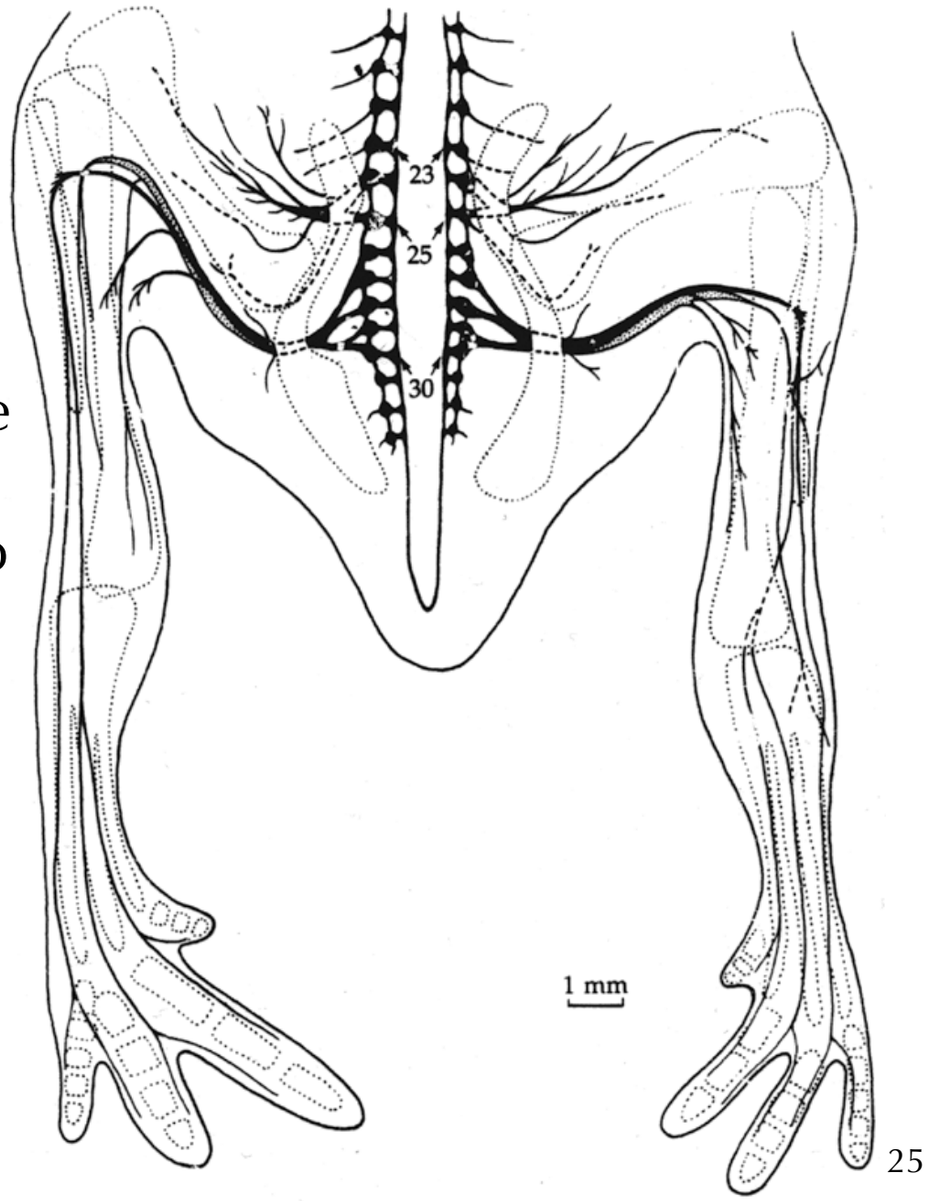
Dorsal root ganglia
Semaphorin3A-secreting COS cells

Retinal growth cone turns away from contact with a bead whose surface is coated with a repellent ephrin-A2.

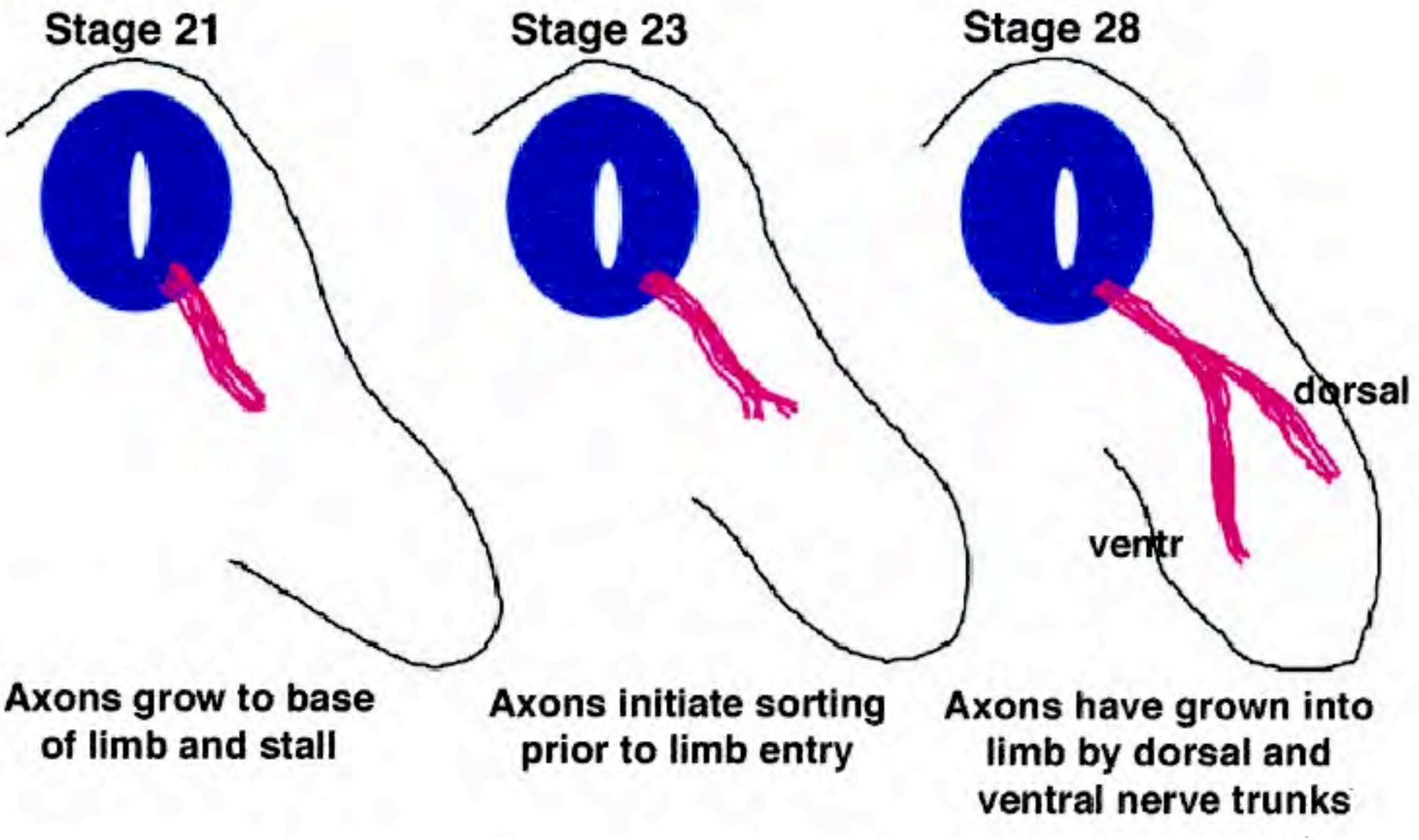


Vertebrate PNS: Innervation of the limb muscles

Major nerve trunks exit the spinal cord, and segregate at the base of the limb into nerves innervating dorsal and ventral targets.



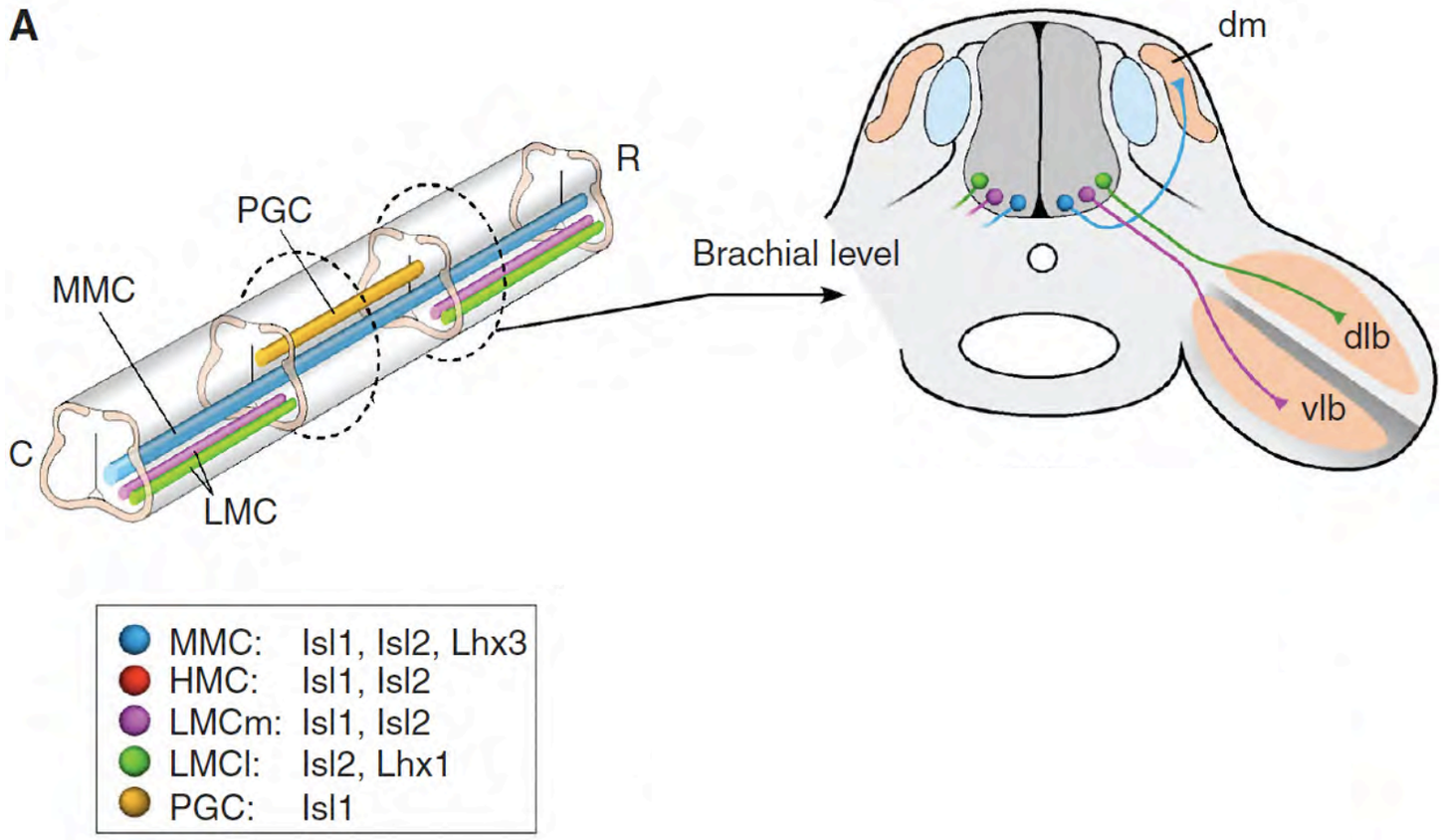
Peripheral axons extend in loose bundles to the base of a limb. Then, axons sort out and diverge toward their respective dorsal or ventral targets (muscle or skin).



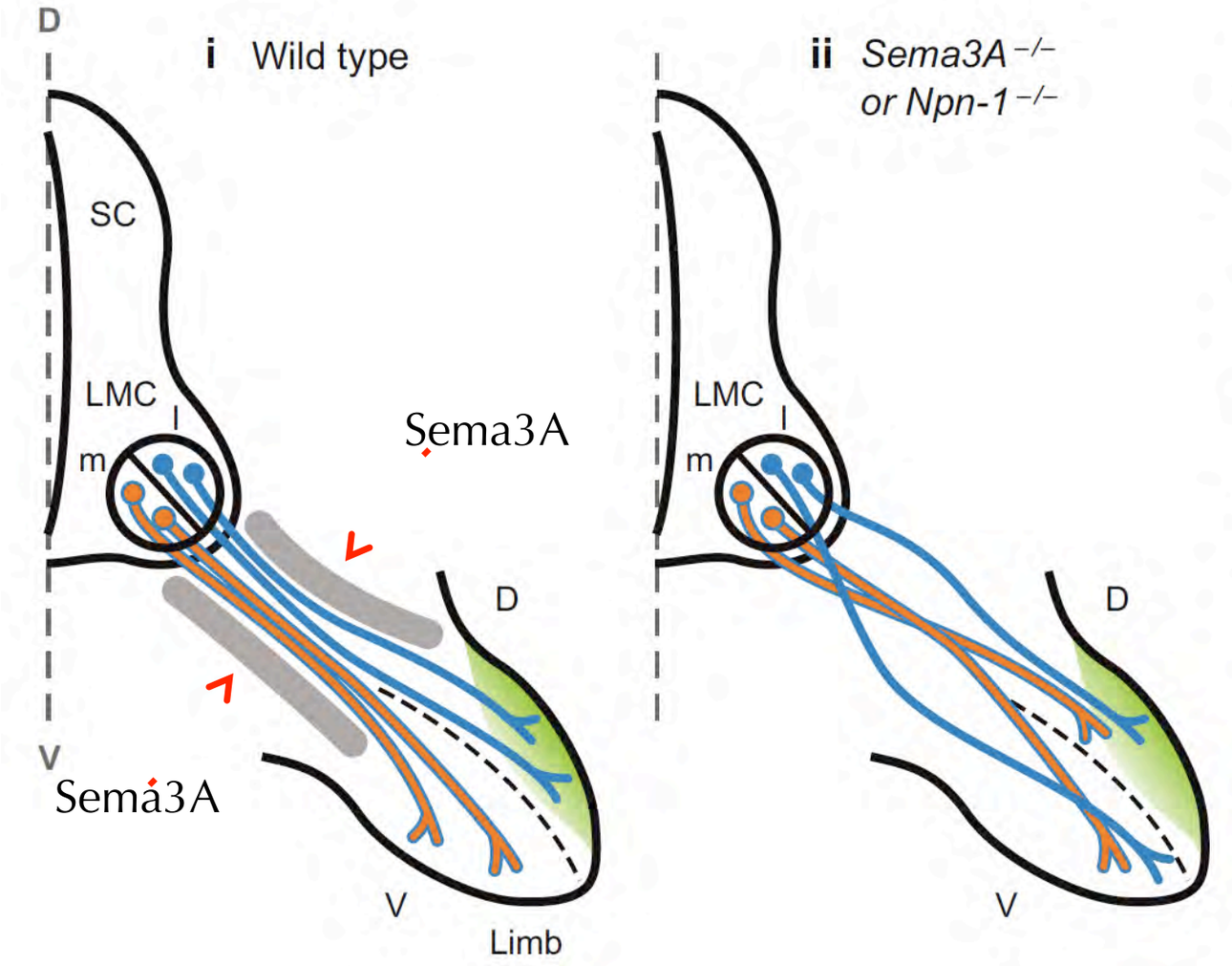
•Motorneurons of LMCl (green) innervate dorsal limb muscle

•Motorneurons of LMCm (purple) innervate ventral limb muscle

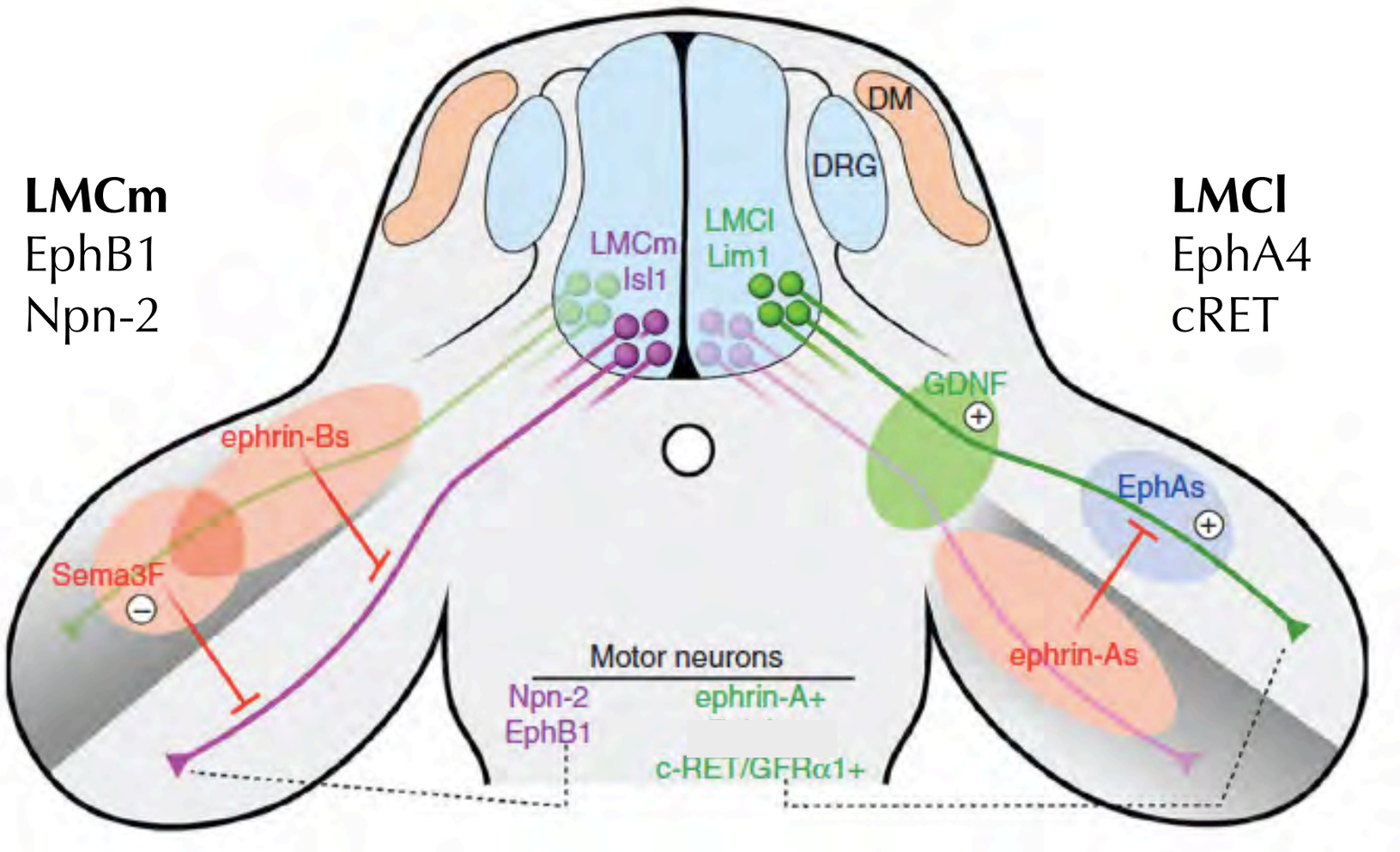
•Motorneurons of MMC (blue) innervate axial muscle



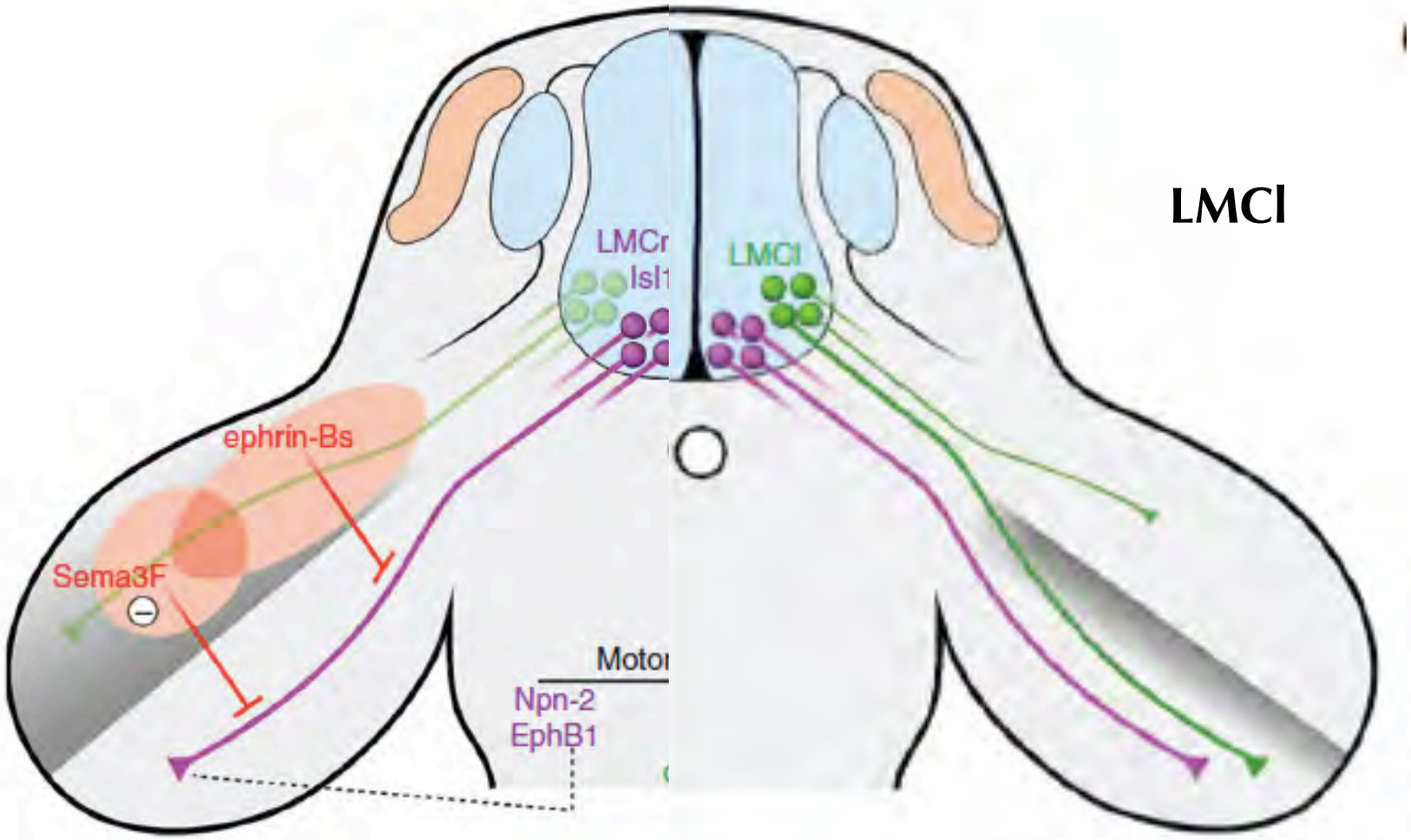
Expression of Sema3A by limb tissues keeps axons from straying laterally while they sort out and enter limb.



Guidance of motor axons into dorsal vs ventral limb is regulated by motor neuron expression of EphA EphB and Npn receptors and by limb mesenchyme expression of ligands ephrinA, ephrinB and Sema3F.



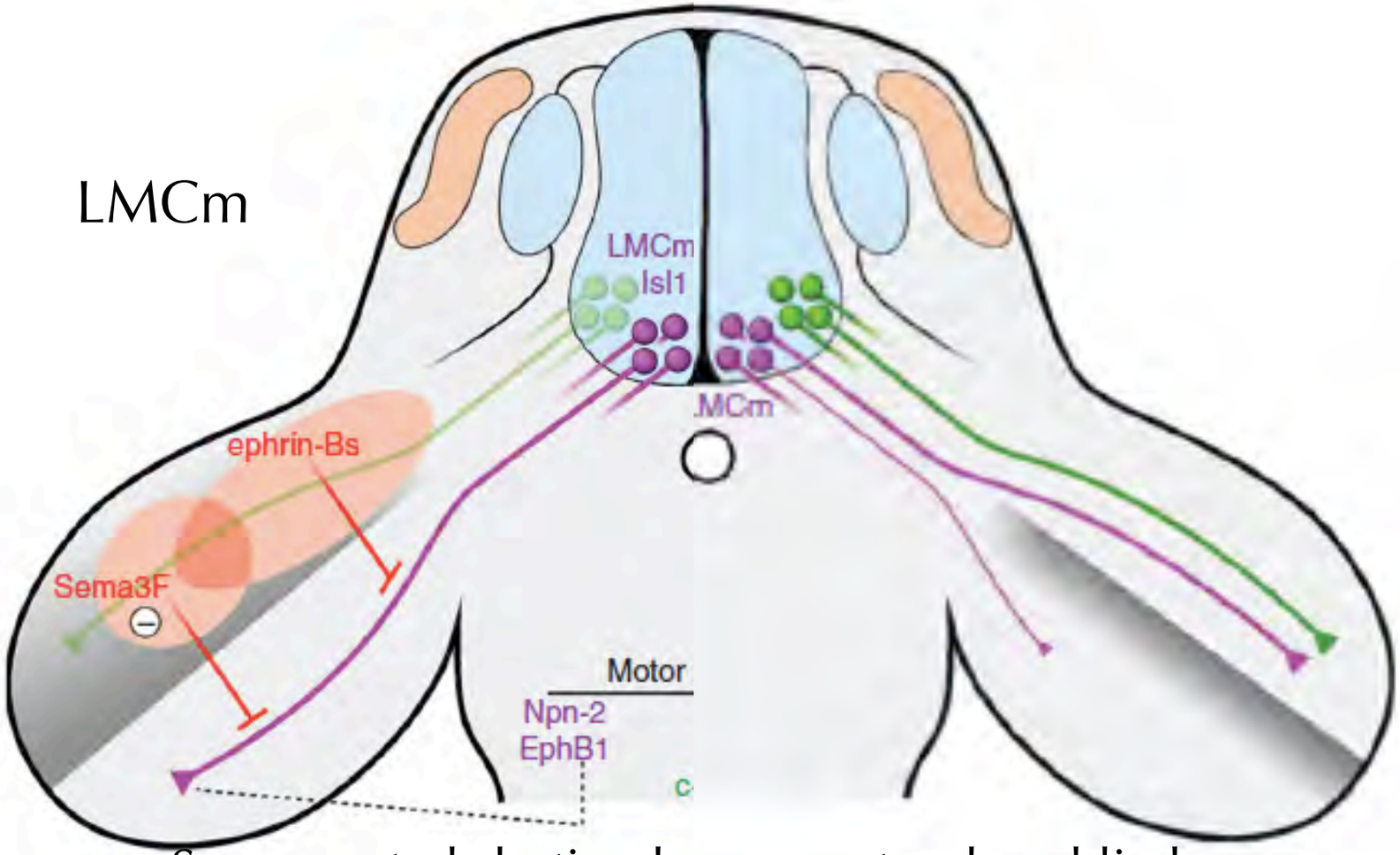
If LMCl neurons do not express EphA4 or cRet or
if limb tissue does not express ephrinA or GDNF



Some dorsal-destined axons enter ventral limb

If LMCm neurons do not express Npn-2 or EphB1 or
If dorsal limb tissue does not express Sema3F or ephrin-B

LMCm

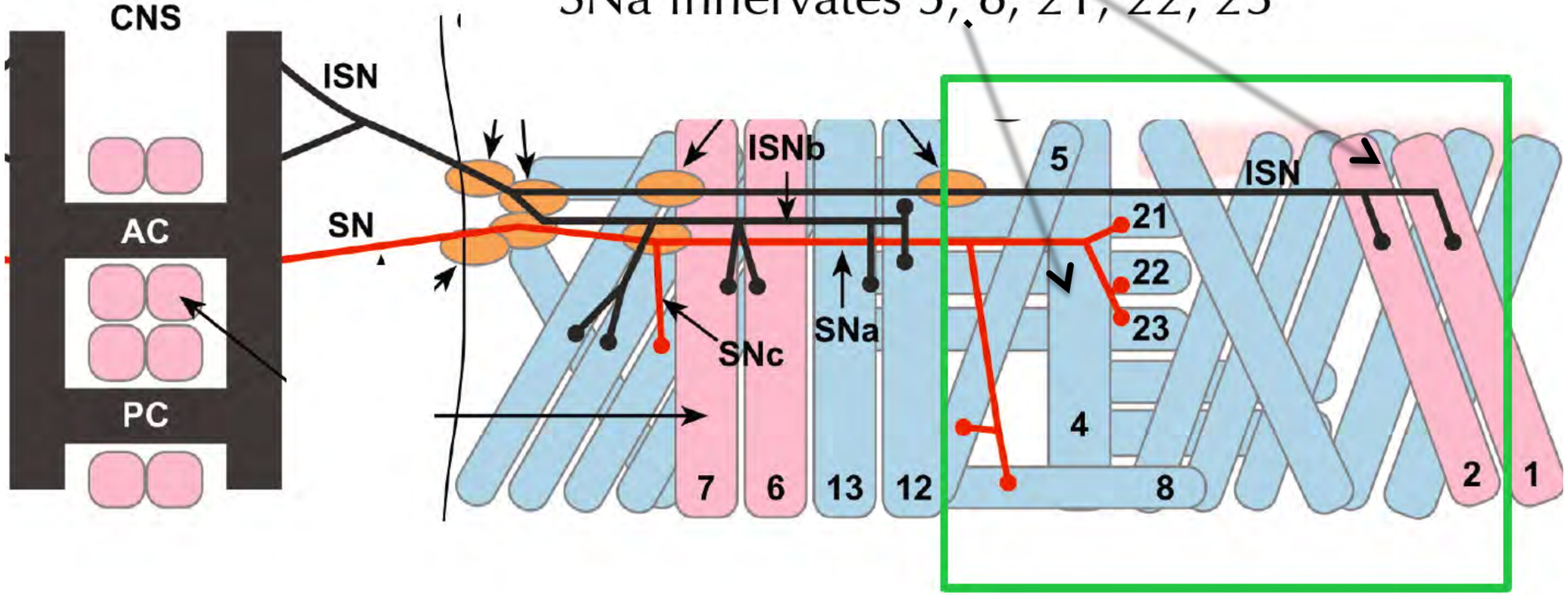


Some ventral-destined axons enter dorsal limb

Drosophila peripheral nerve innervation of muscles

ISN innervates 1, 2

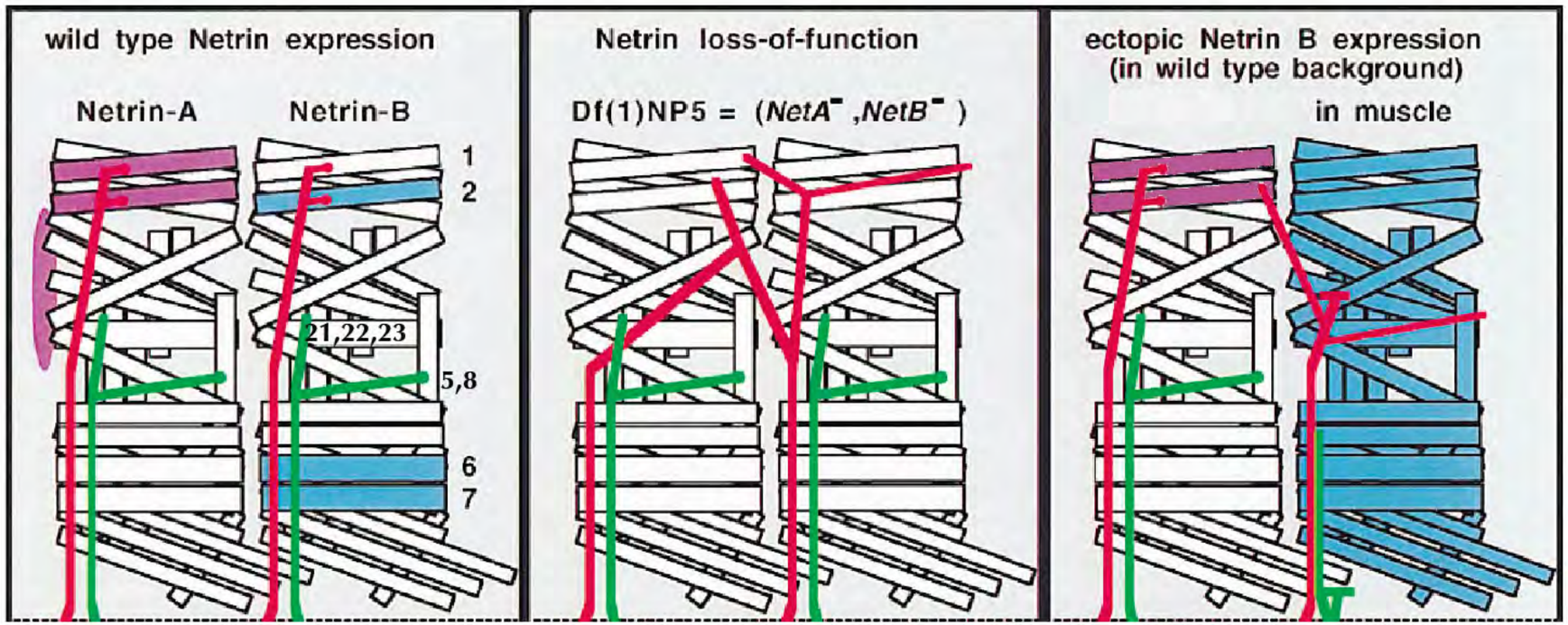
SNa innervates 5, 8, 21, 22, 23



Attraction and repulsion by muscle-derived netrin regulates target innervation.

ISN = Attracted by netrin; Innervate 1, 2

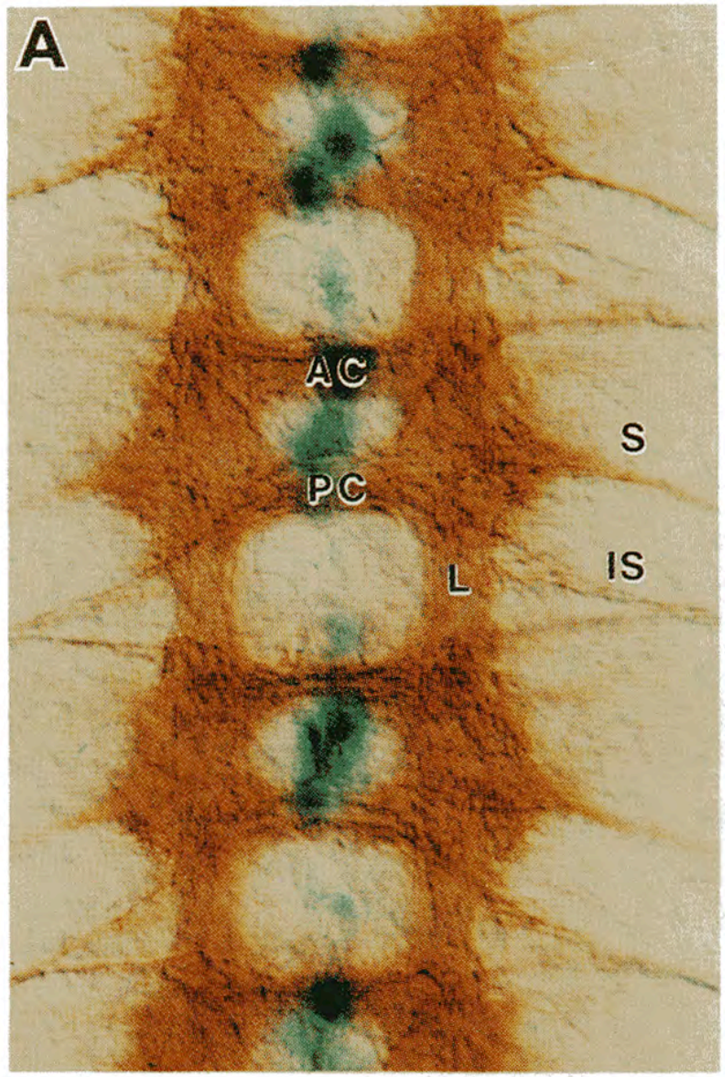
SNa = Repelled by netrin; Innervate 5, 8, 21, 22, 23

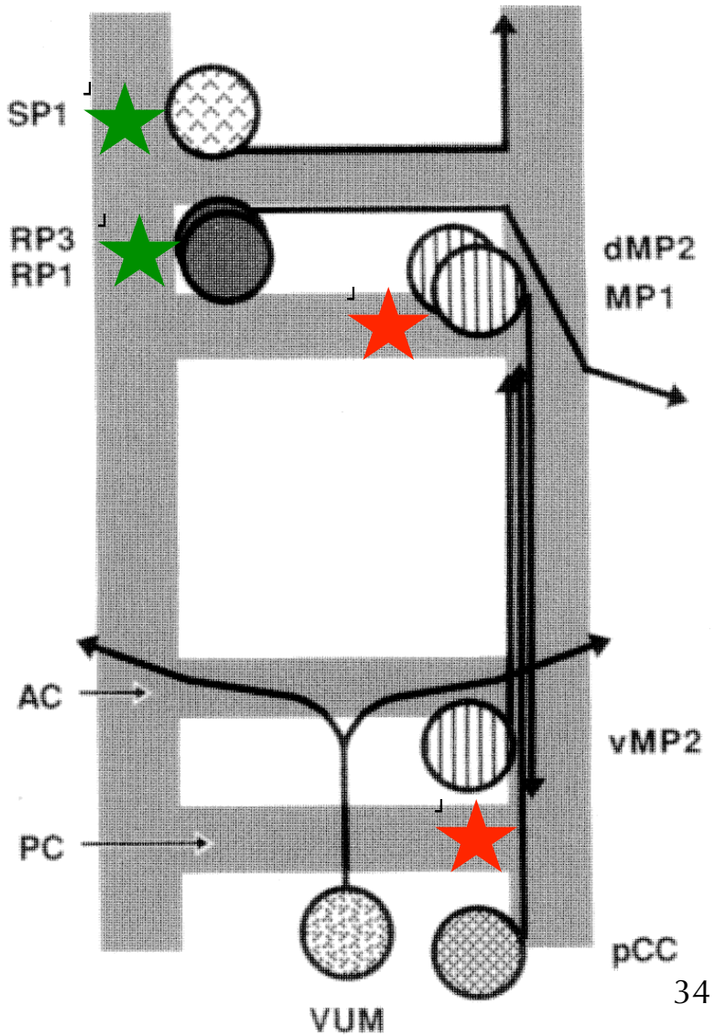


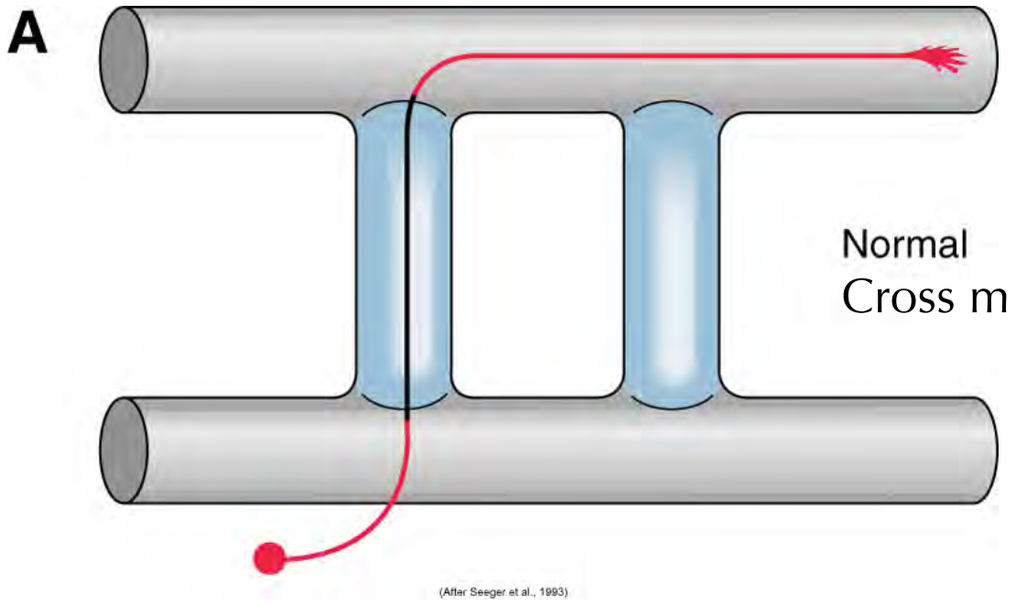
ISN does not innervate 1,2
SNa innervates normally

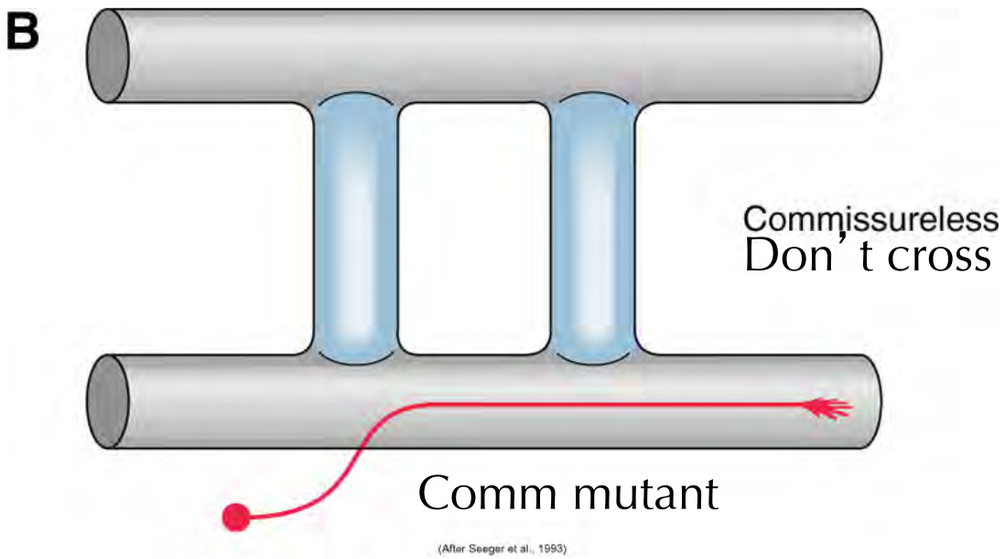
ISN innervates extra targets
SNa stalls, binds to ISN

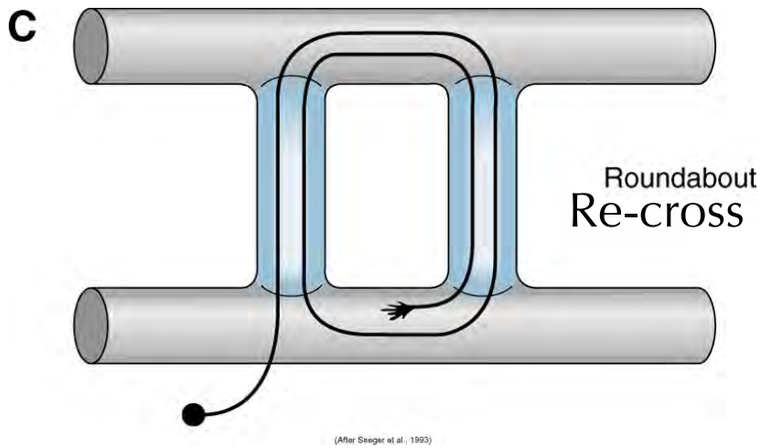
Drosophila CNS: Some axons cross the midline, while some axons never cross the midline.



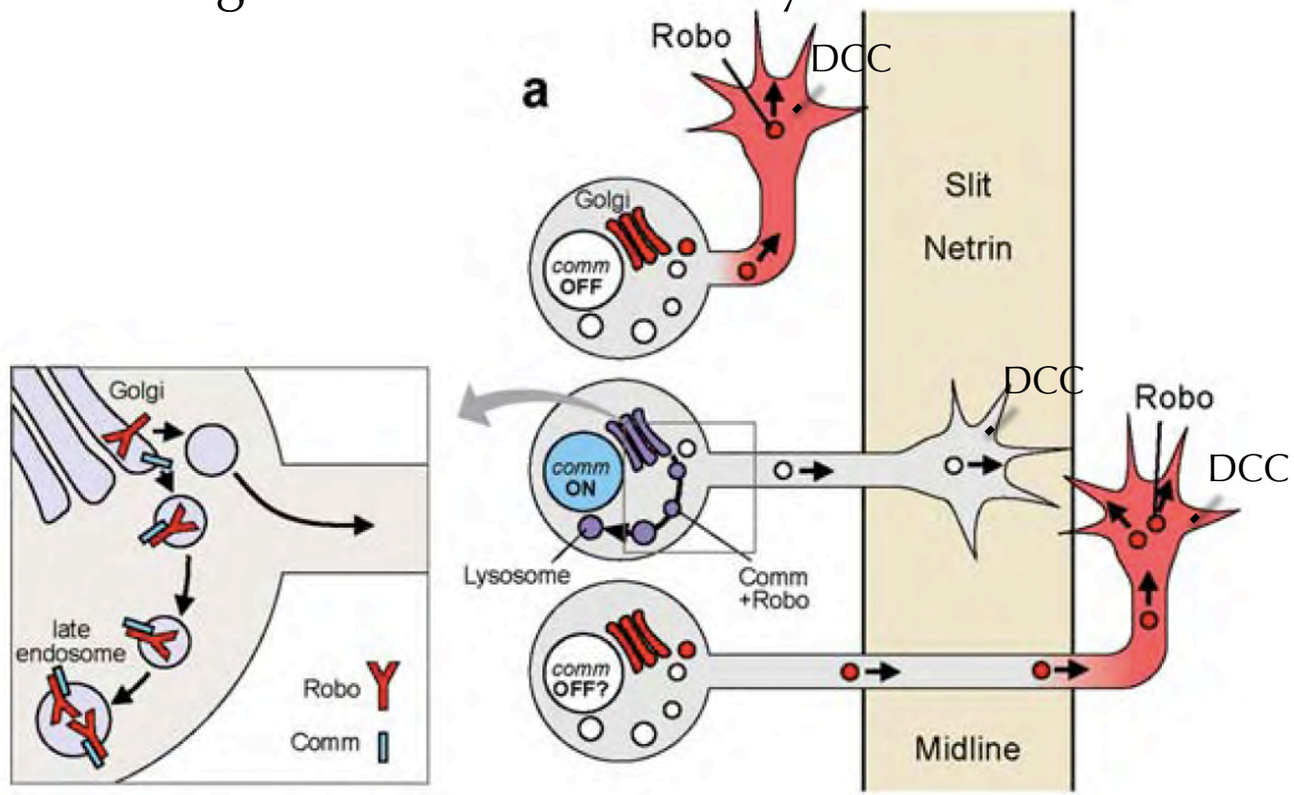


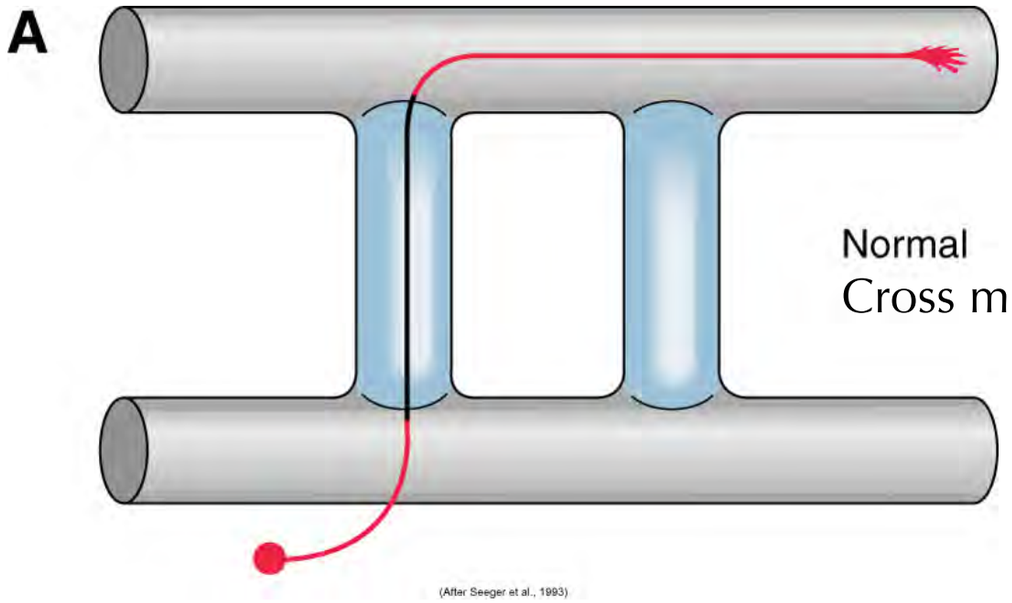

A
Normal
Cross midline
(After Seeger et al., 1993)

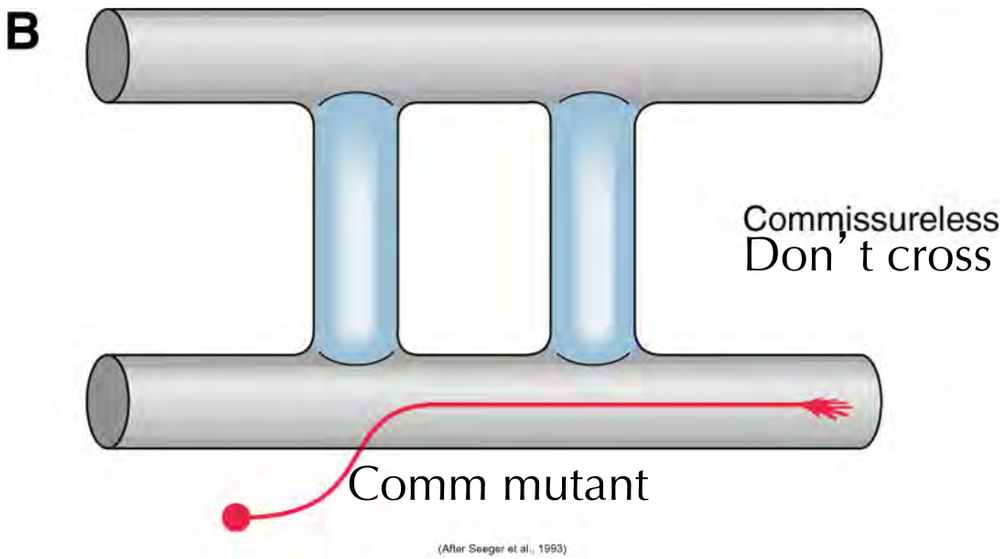

B
Commissureless
Don’ t cross
Comm mutant
(After Seeger et al., 1993)


C
Roundabout
Re-cross
(After Seeger et al., 1993)
Robo mutant

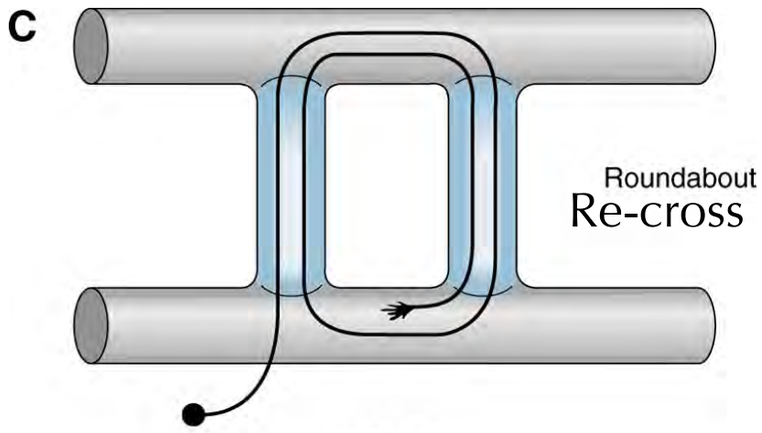
- Midline cells produce netrin, an attractant, and slit, a repellent.
- The robo protein is a receptor for slit.
- The DCC receptor mediates attraction to netrin at the midline.
- Growth cones that never cross the midline always express robo.
- Growth cones that cross express robo only after they cross.
- Comm is a protein that blocks insertion of robo onto the surface of commissural growth cones until they cross the midline.





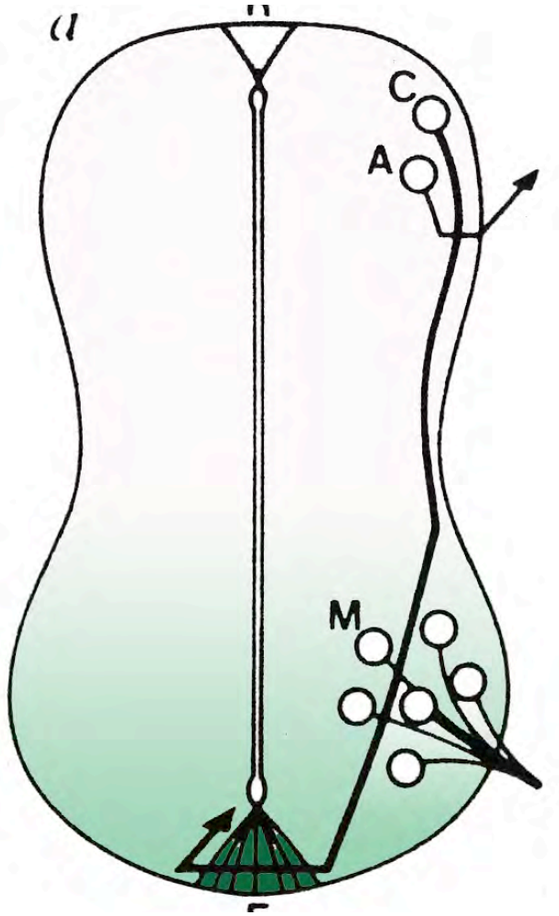


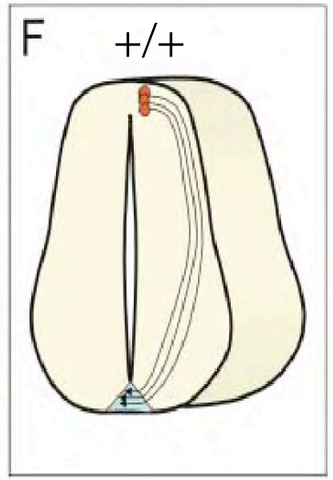
Always express Robo

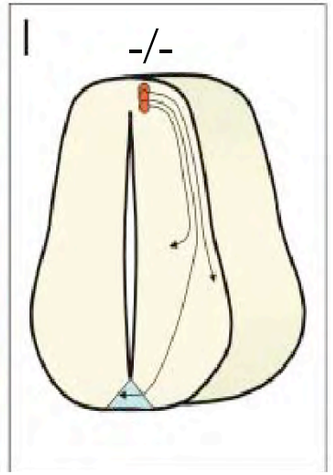


Never express Robo activity

•Spinal commisural neurons are interneurons that project to the contralateral spinal cord. **Netrin** is a guidance cue secreted by floor plate cells. It establishes a gradient that attracts commissural axons toward the floor plate.
•When **netrin** is missing or commisural axons do not express its receptor DCC, many commissural axons do not reach the midline.

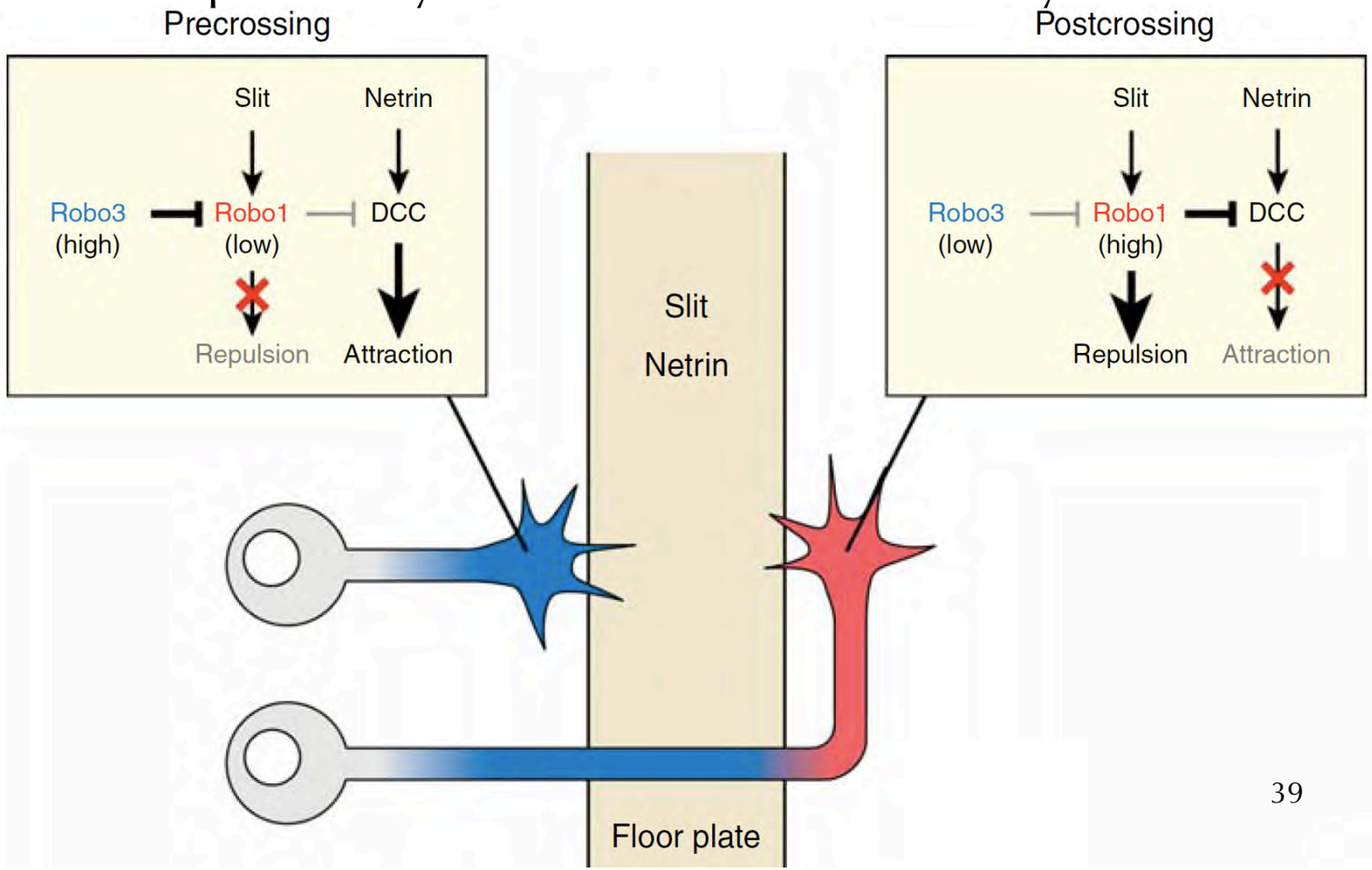




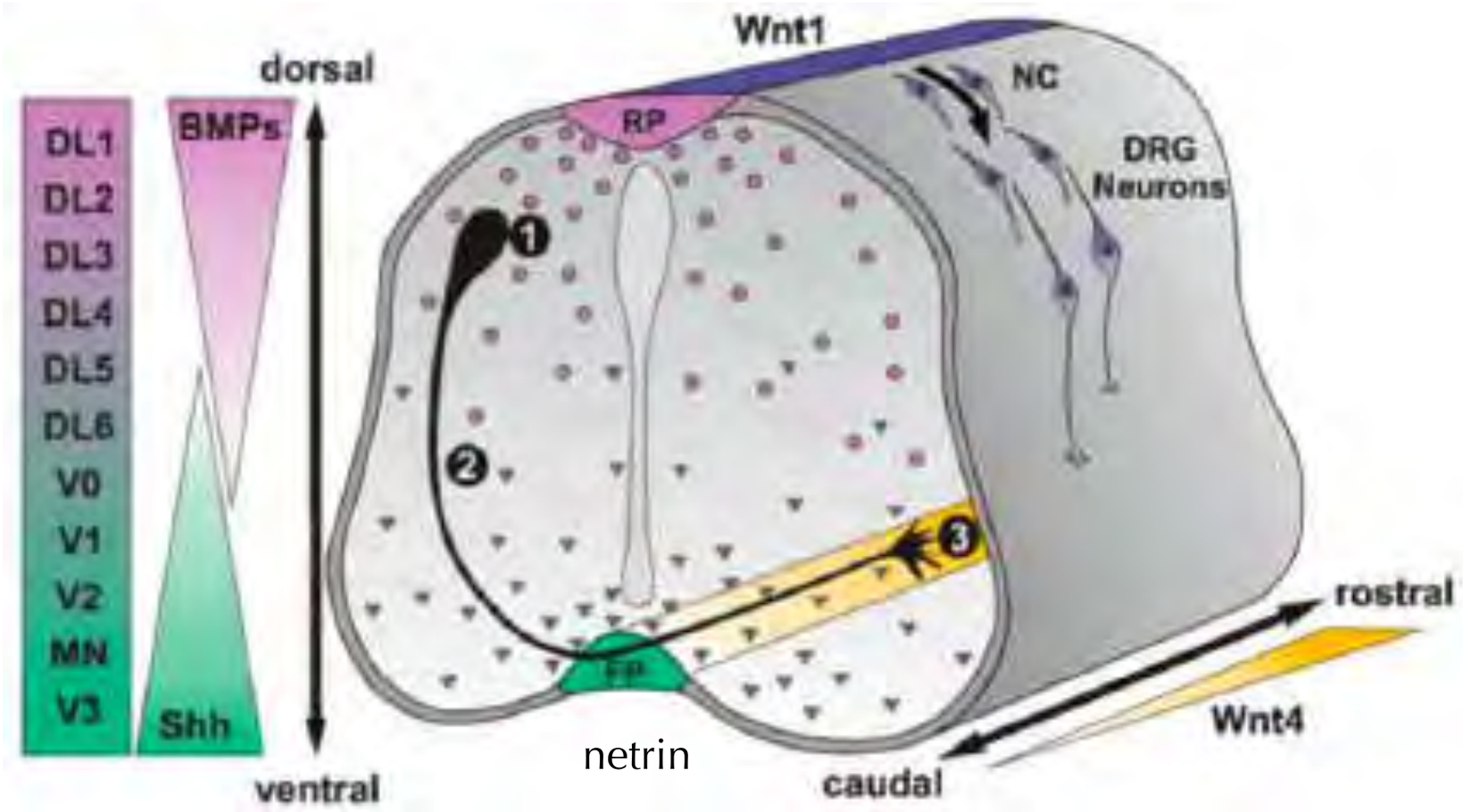


Vertebrates

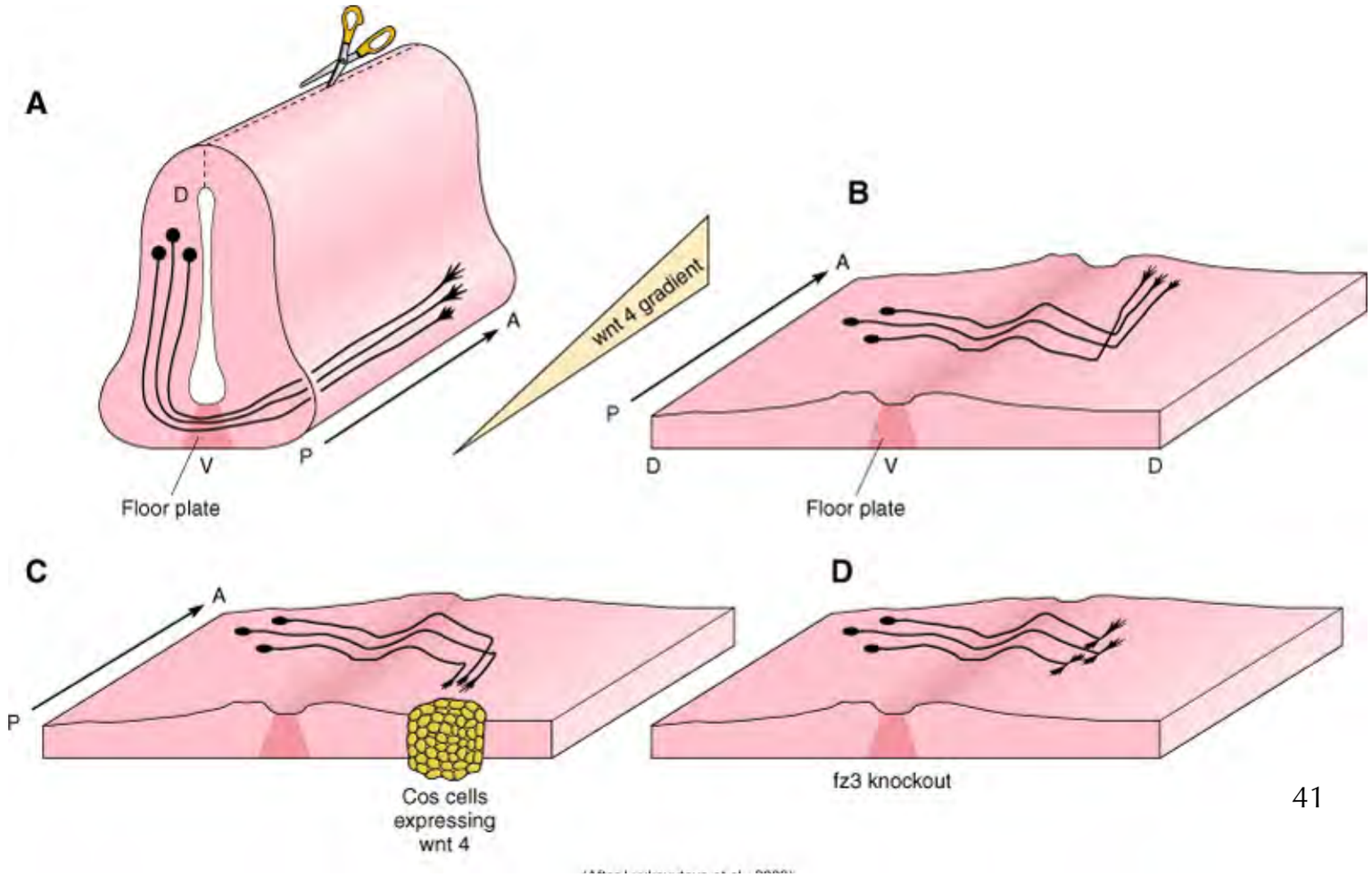
Vertebrates express netrin, slit, DCC and robo1, but not comm.
Robo3 blocks robo1 as a slit receptor.
After crossing Robo3 is downregulated, allowing robo1 to mediate repulsion by slit and inhibit DCC activity.



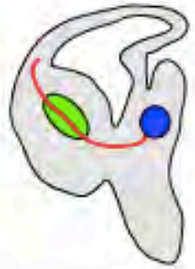
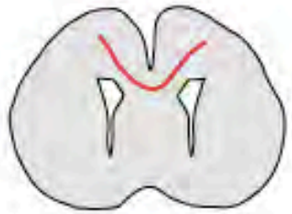
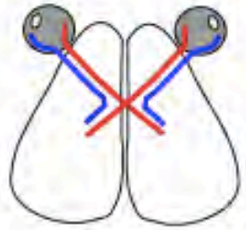
Molecules that function in determining neuronal type earlier in development are also axon guidance cues during growth cone navigation.
BMPS, Shh, Wnt1, Wnt4



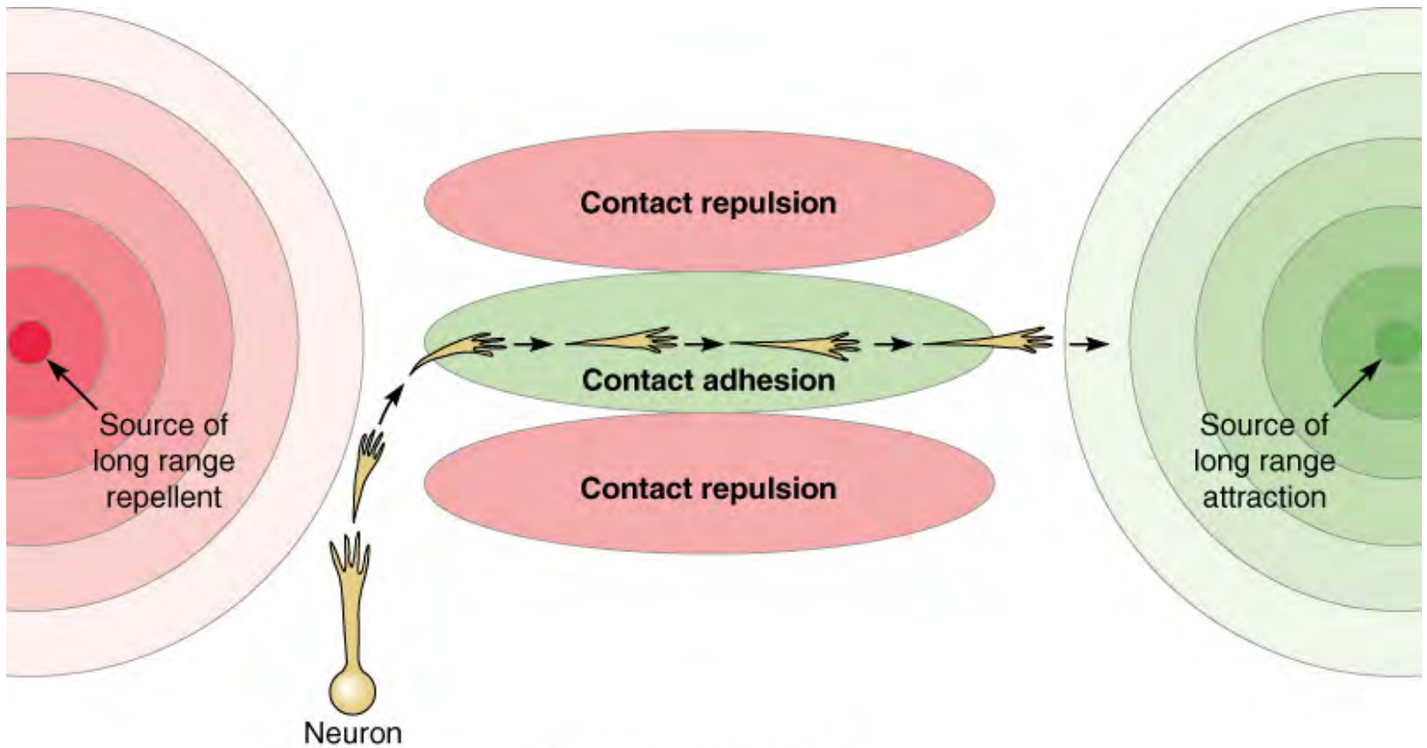
- In vertebrate spinal cords commissural axons cross the ventral midline and then turn toward the brain.
- A gradient of the guidance cue wnt4 attracts the turn toward brain.
- Axons lacking the wnt4 receptor fail to turn.



Conservation of guidance cues and receptors across regions

	Spinal cord	Brain		
				
Guidance cue/ receptor	**dI1 commissural axons**	**Thalamo-cortical axons**	**Corpus callosum**	**Optic chiasm**
Netrin/Dcc	+	+	+	+
Slit/Robo	+	+	+	+
Sema3/neuropilin	+	+	+	+
Sema6/plexin	+	+	?	+
Ephrin/Eph	+	+	+	+
Wnt/Frz3 or Ryk	+	+	+	+
Draxin/Dcc	+	+	+	?

•Growth cone navigation involves interactions with multiple environmental guidance cues.
•Each segment of the navigation pathway involves interactions with a different set of guidance cues.



(After Tessier-Lavigne and Goodman, 1996)

Guidance cues act through a series of steps to regulate growth cone behaviors

Guidance Cue

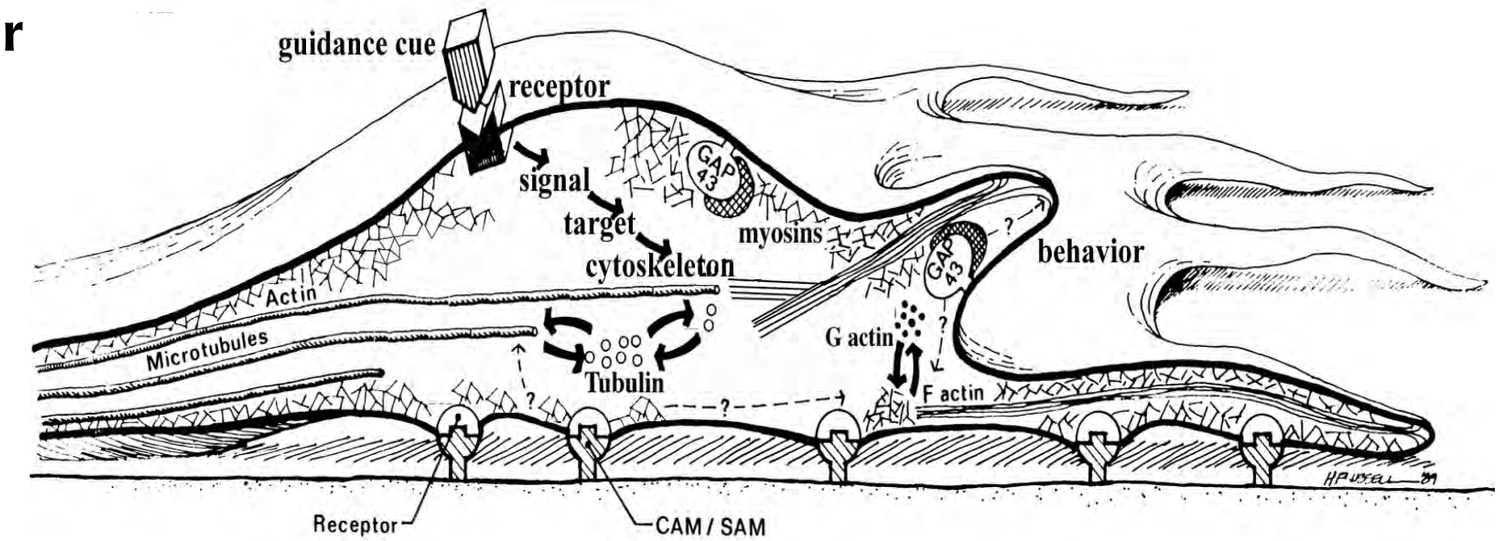
Receptor

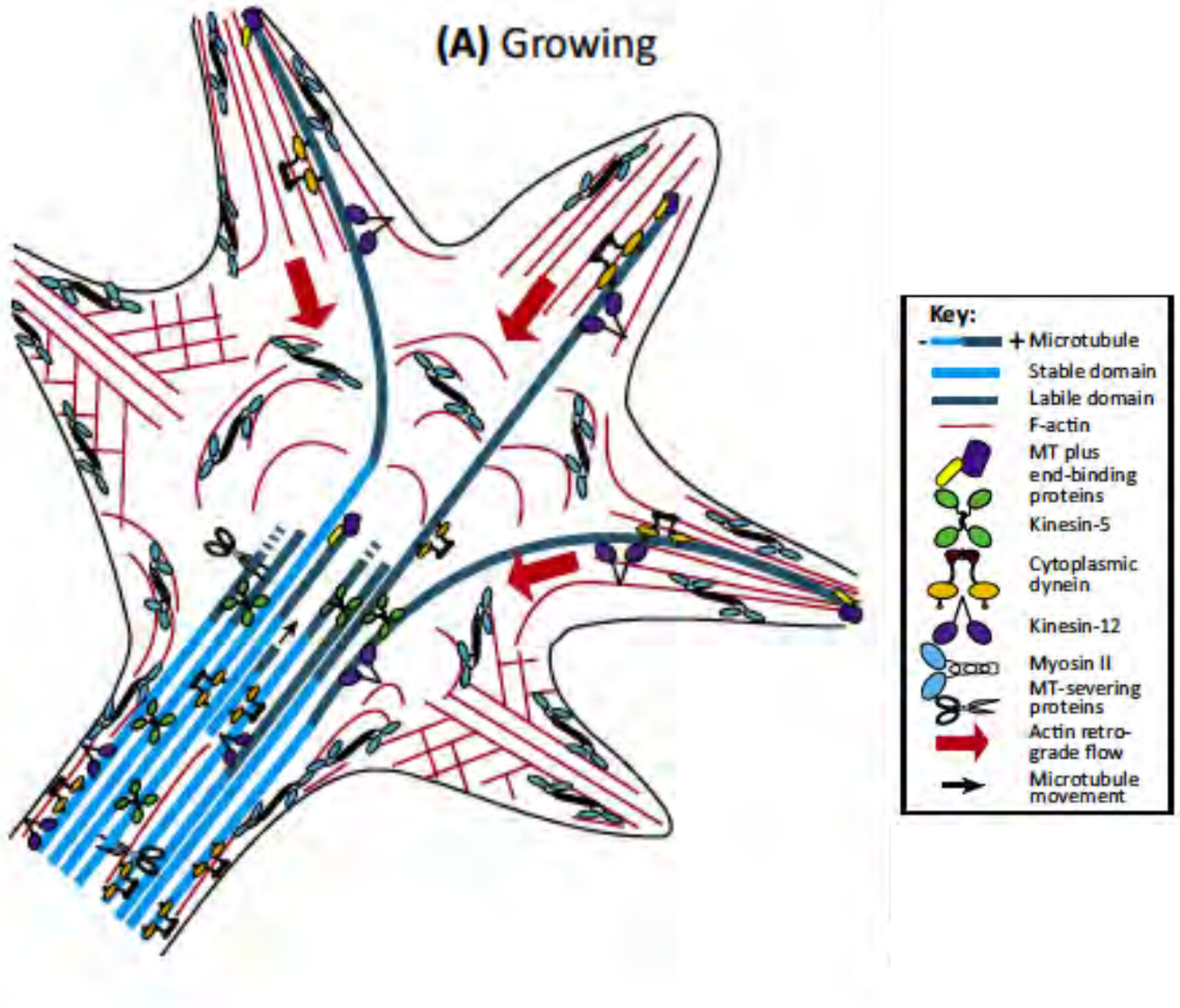
Signal

Target

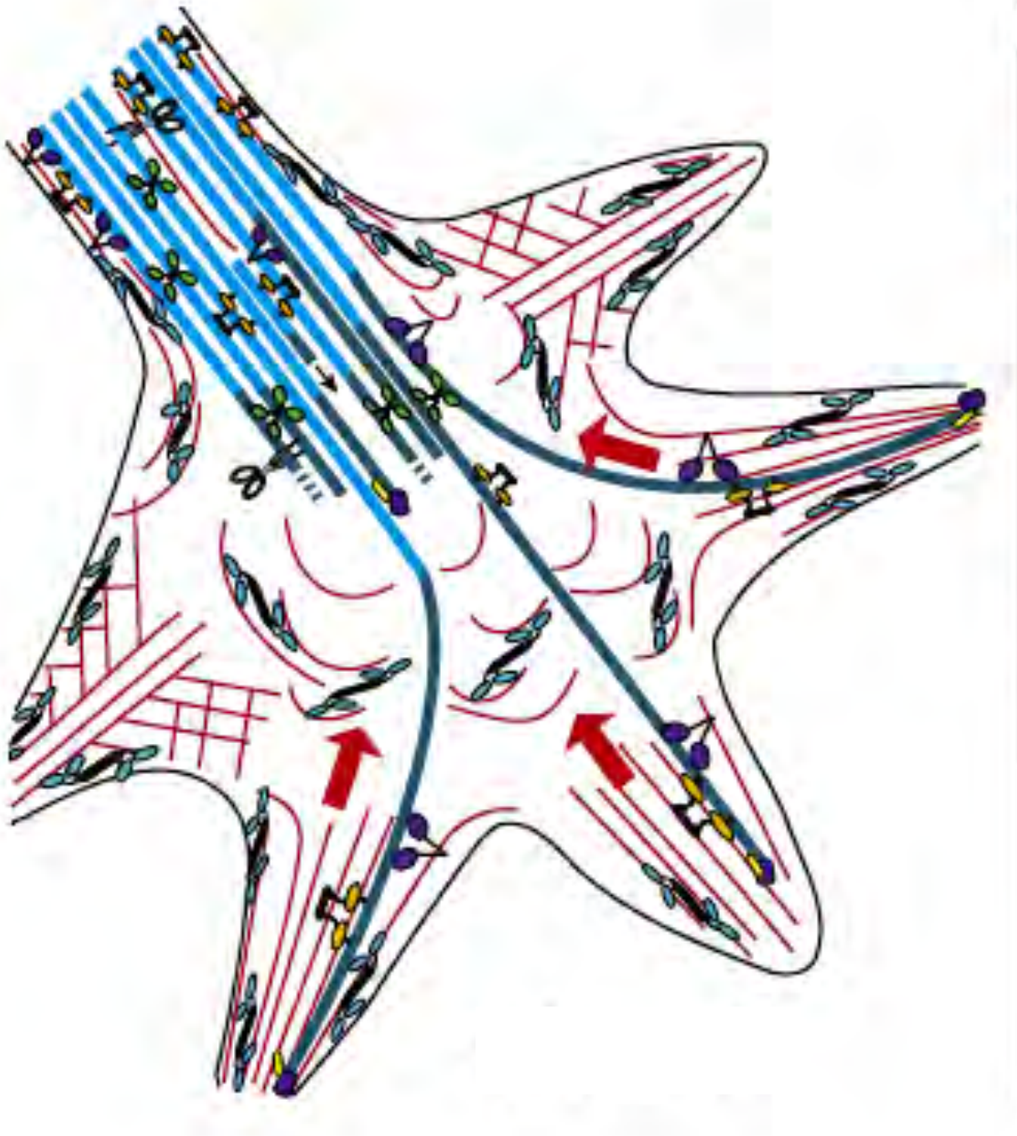
Cytoskeleton

Growth Cone Behavior

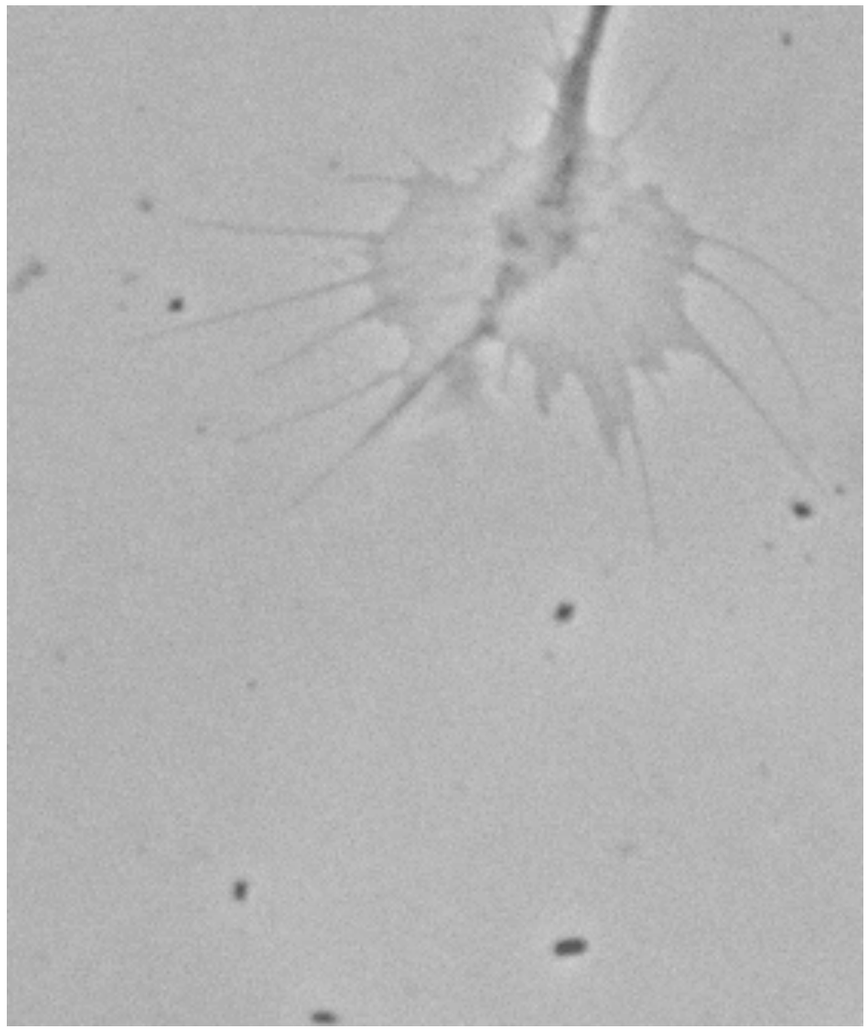




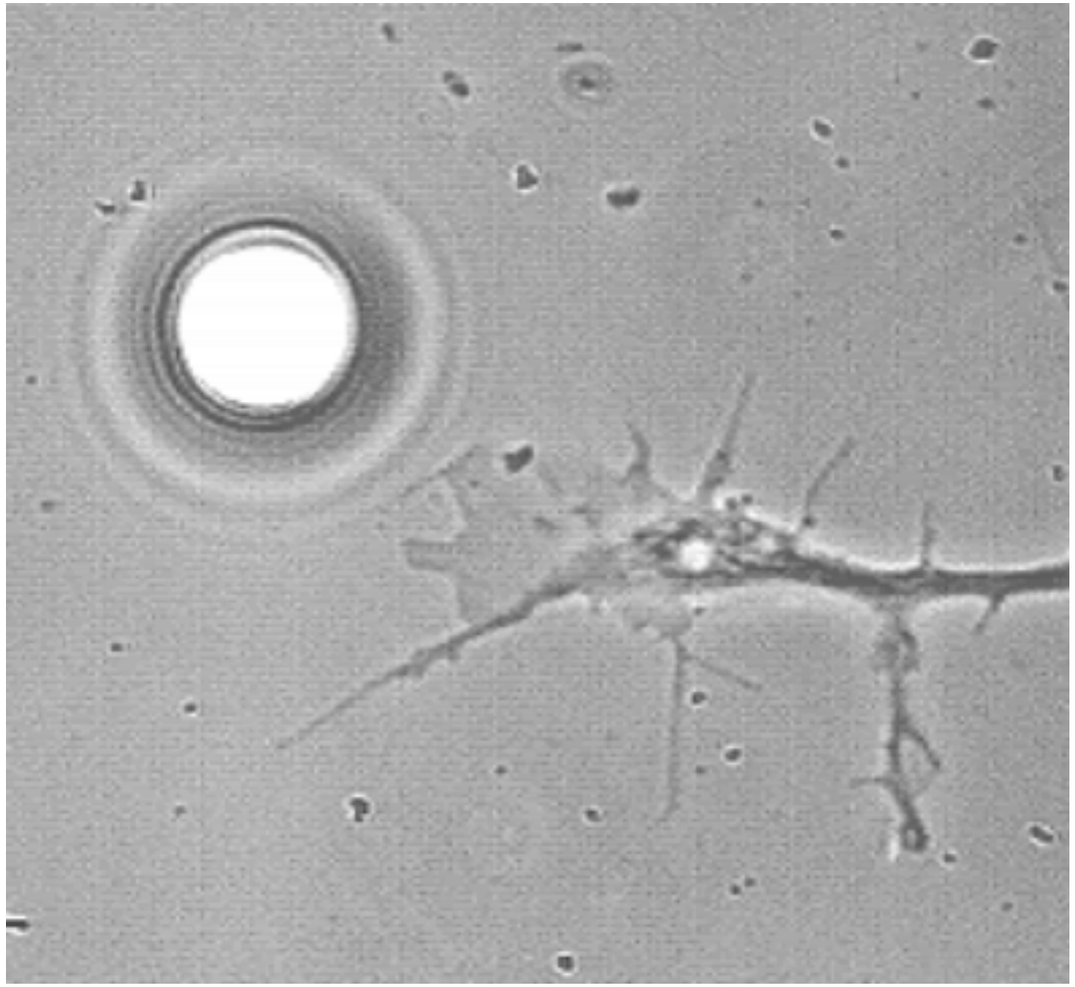
Cytoskeletal activities are symmetric

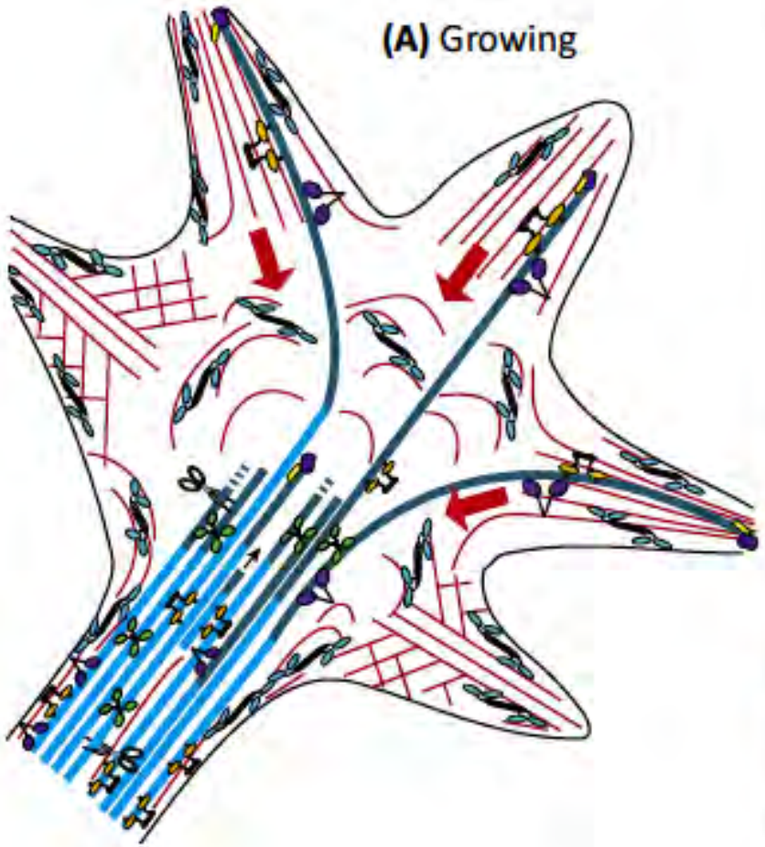


On a homogenous substrate the microtubules and organelles advance straight ahead, although filopodia and lamellipodia explore left and right.

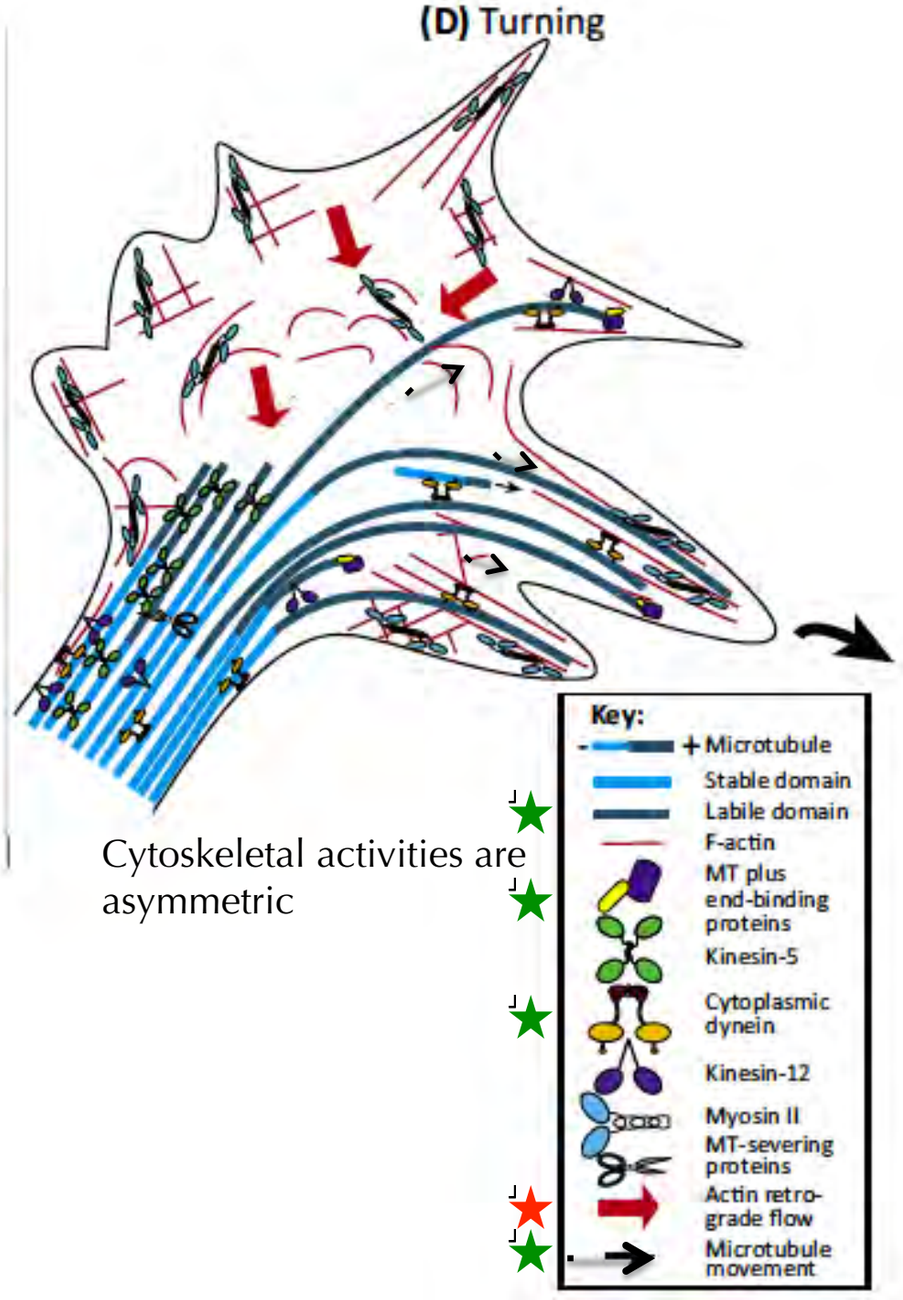


When a filopodium contacts a bead with an attractive surface, the microtubules and organelles shift rapidly to turn the growth cone toward the bead.

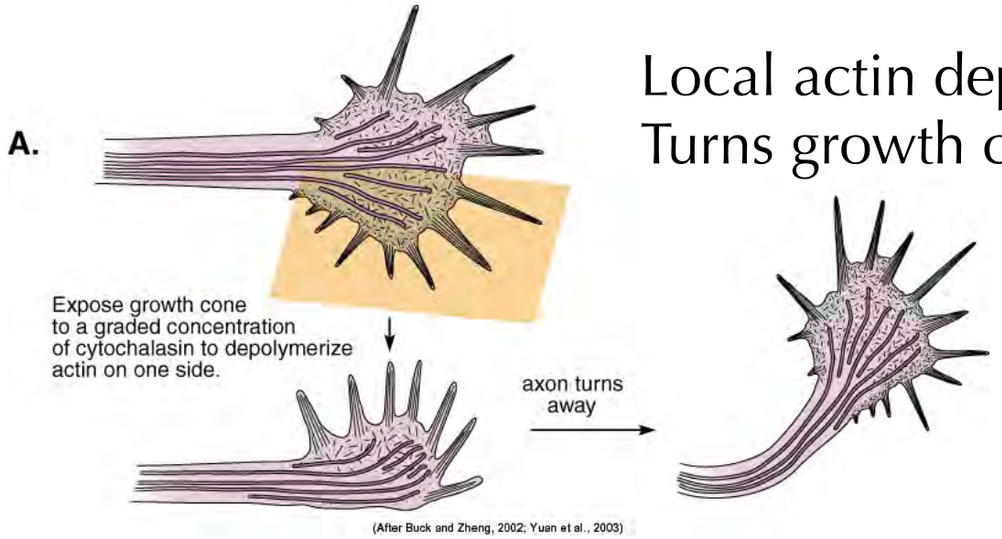




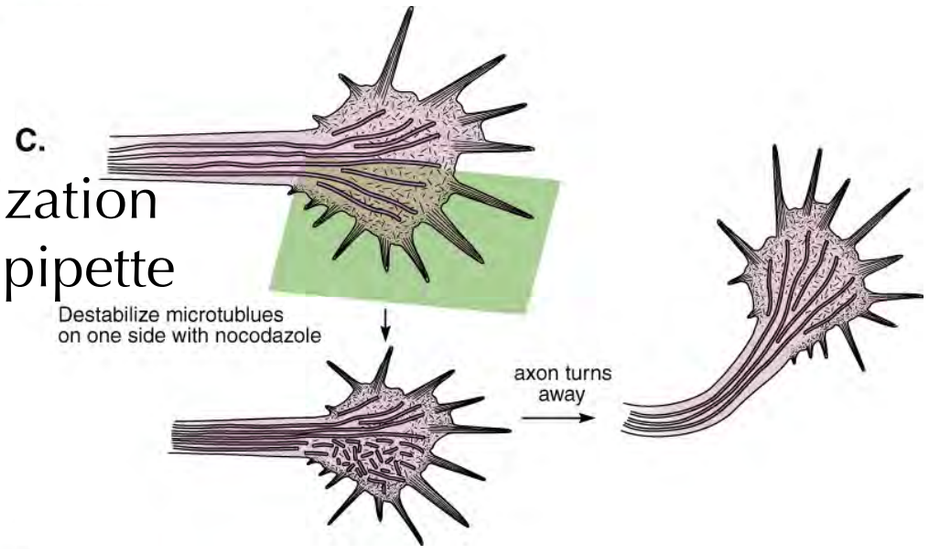
Cytoskeletal activities are symmetric



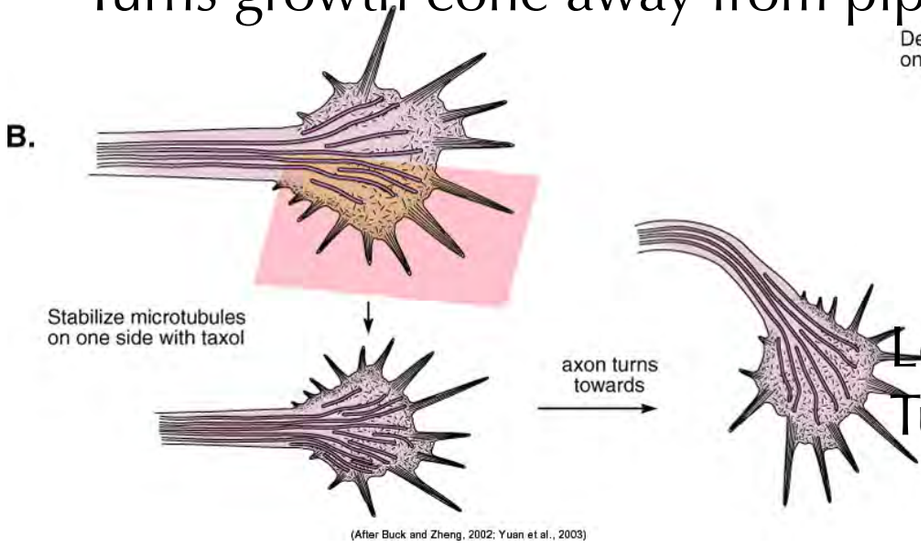
Cytoskeletal activities are asymmetric



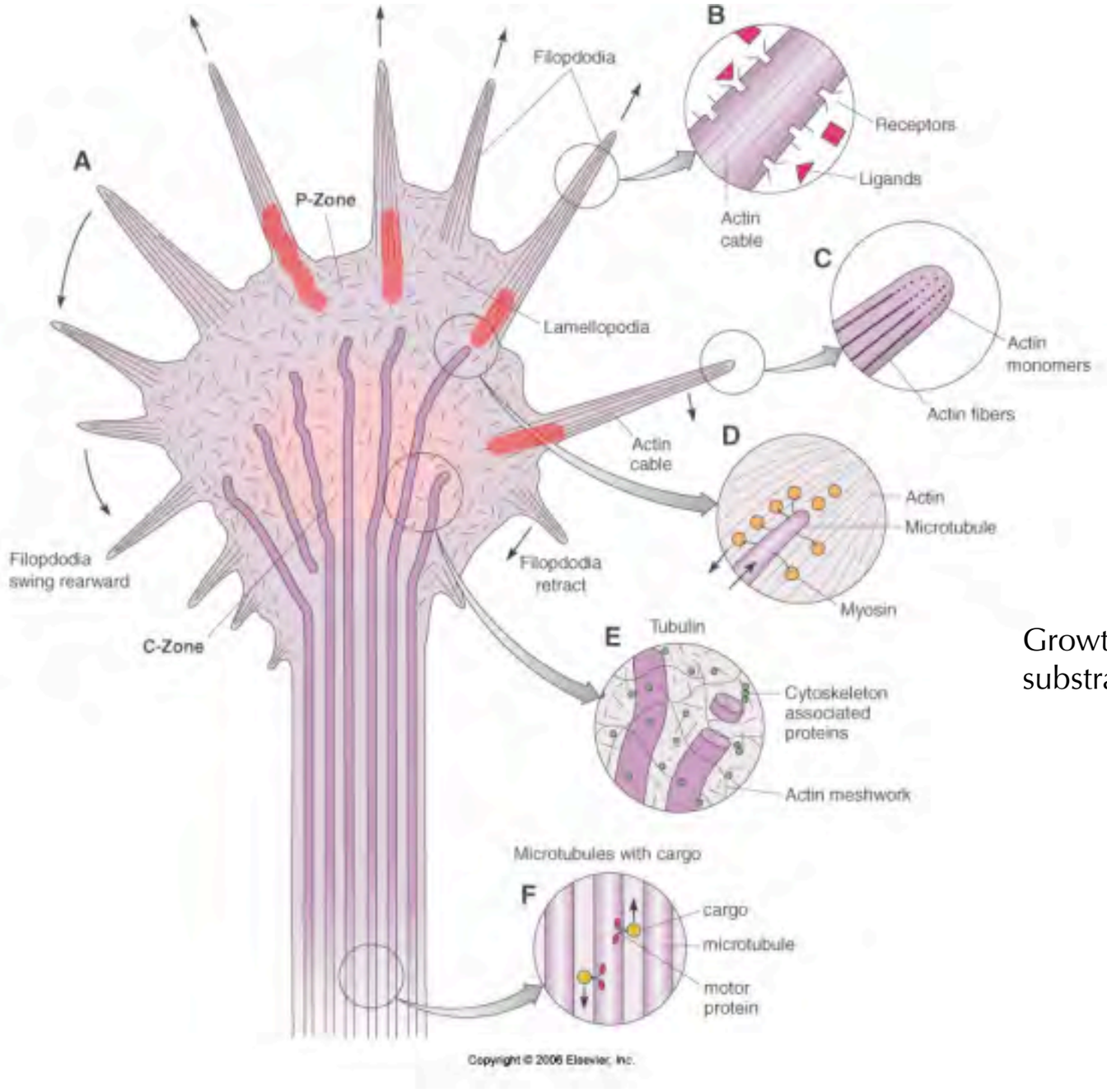
Local actin depolymerization
Turns growth cone away from pipette



Local microtubule depolymerization
Turns growth cone away from pipette

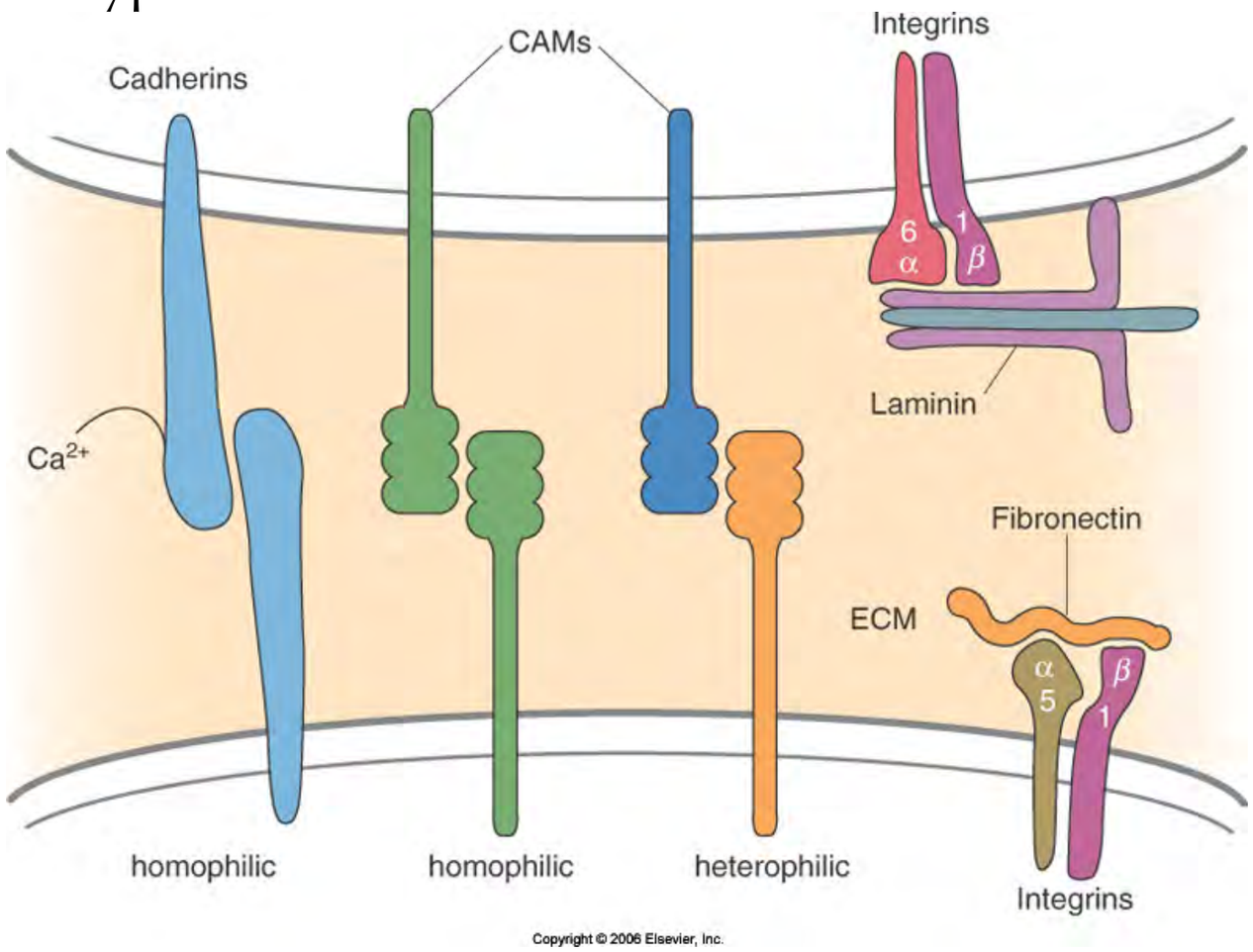


Local microtubule stabilization
Turns growth cone towards pipette



Growth cone-substrate adhesions

Migrating growth cones gain adhesion to their substrate by way of several types of adhesion molecules.



ABPs link F-actin bundles to adhesive sites

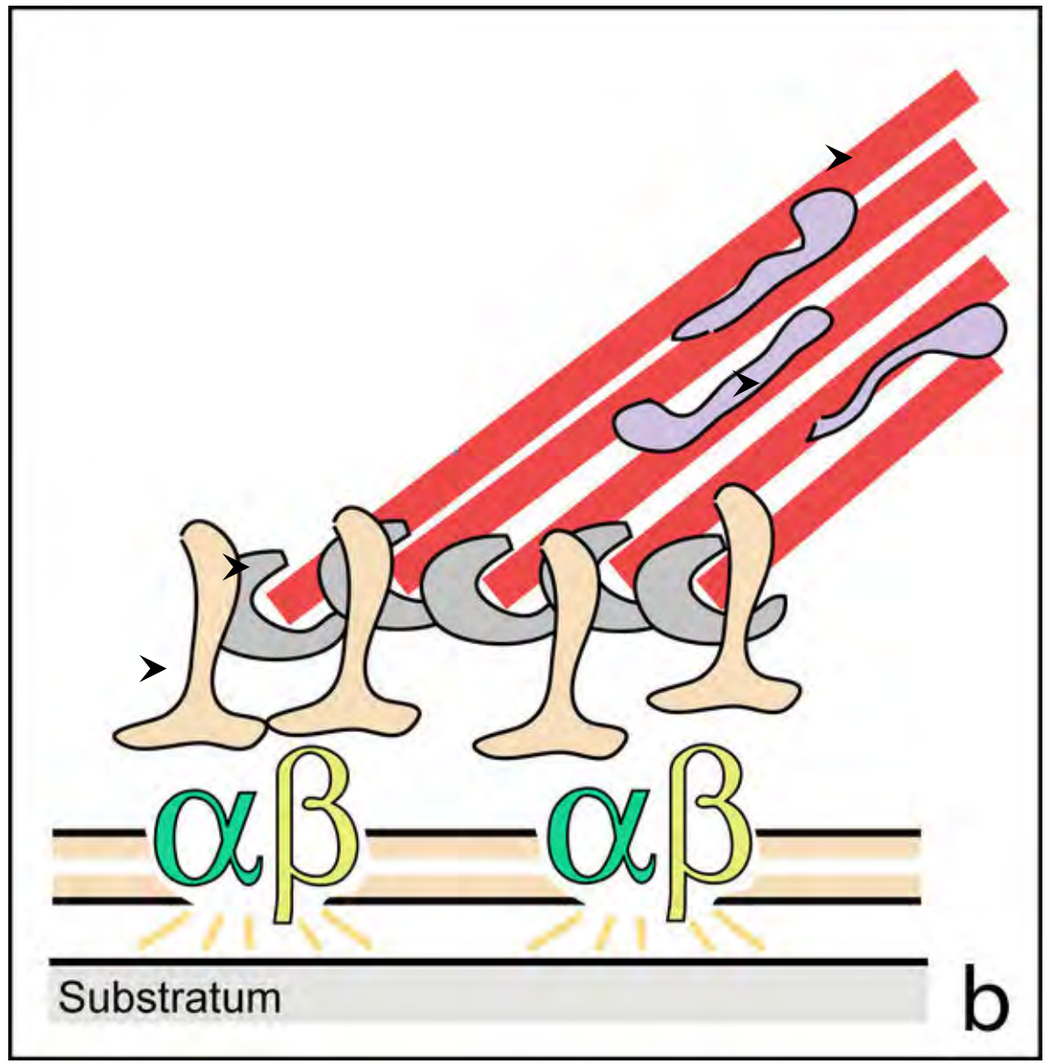
F-actin

alpha-actinin = F-actin linker

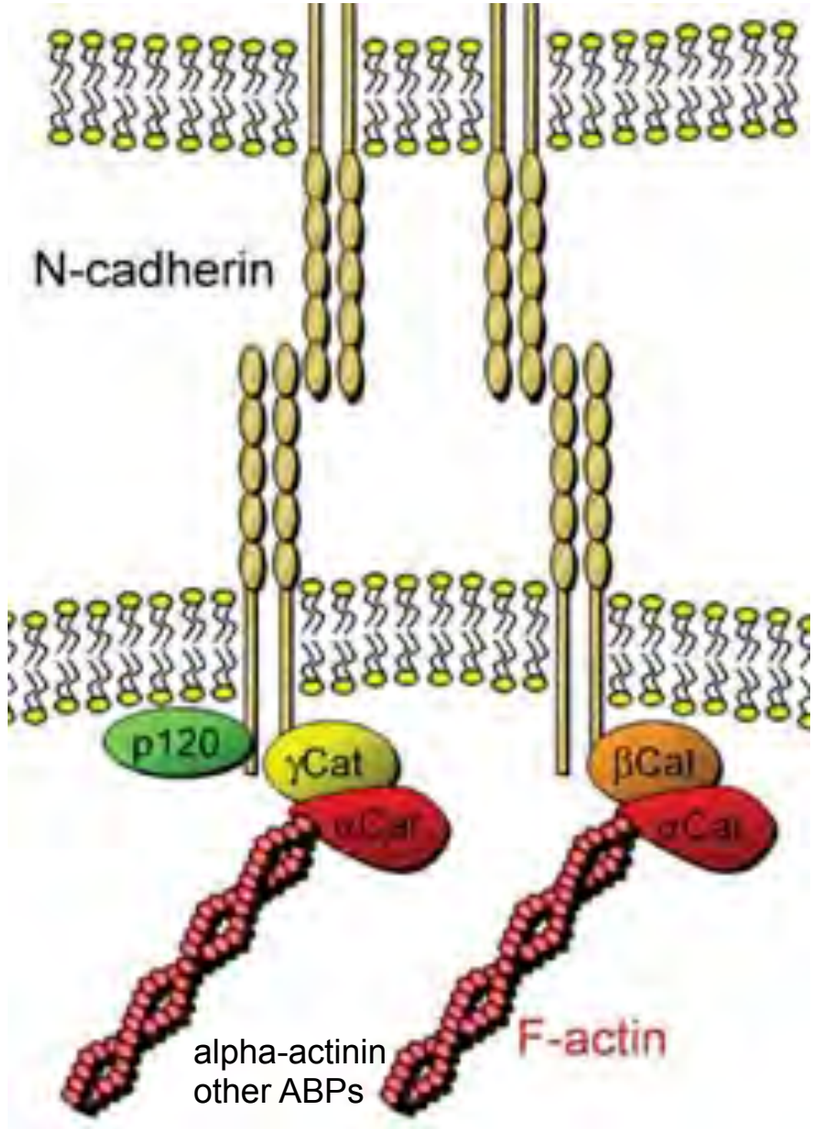
vinculin = actin-integrin linker

talin = actin-integrin linker

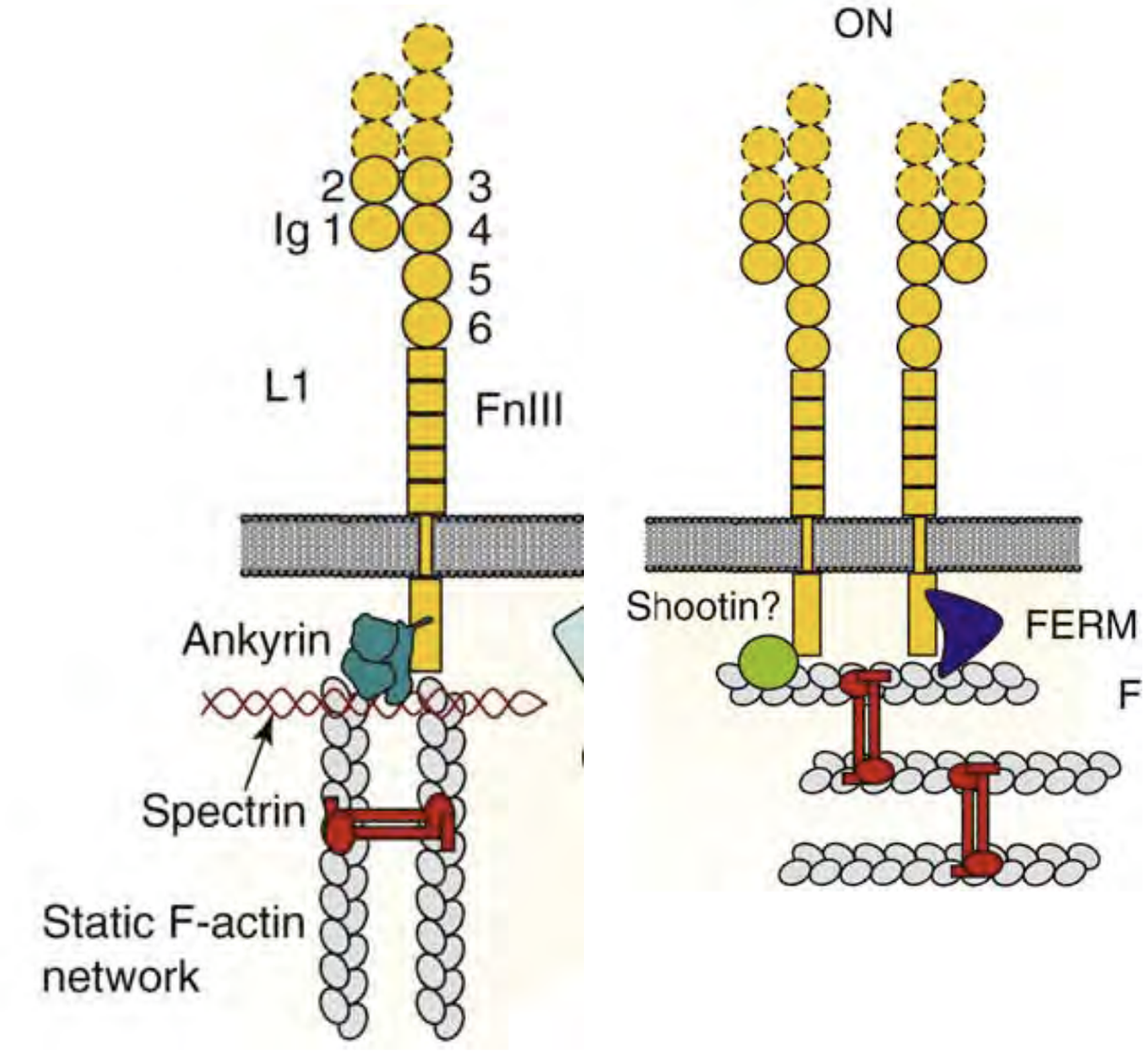
Integrin dimer = adhesion receptor



Catenins mediate linkage of N-cadherin to F-actin

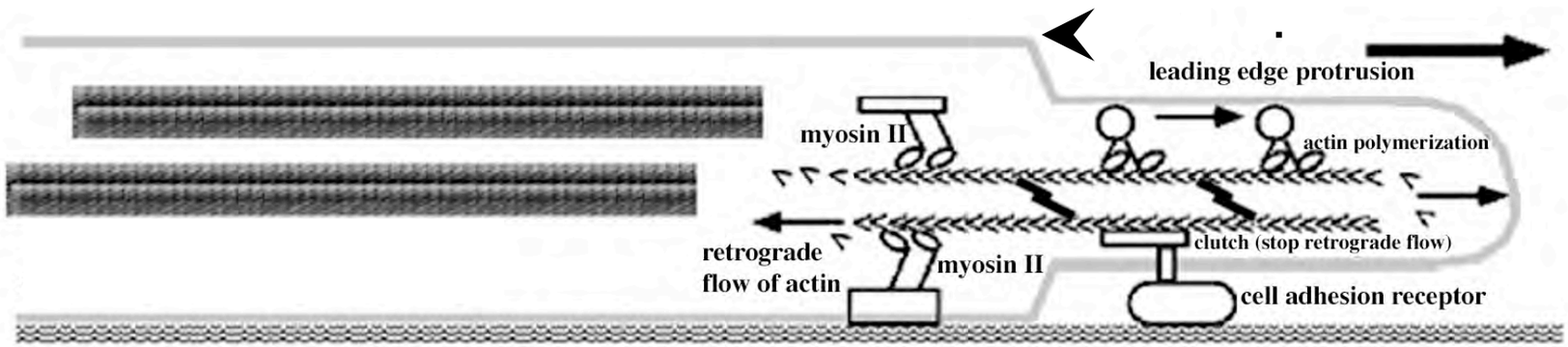


L1 and other IgCAM members link to actin via ankyrin, spectrin and FERM domain proteins.

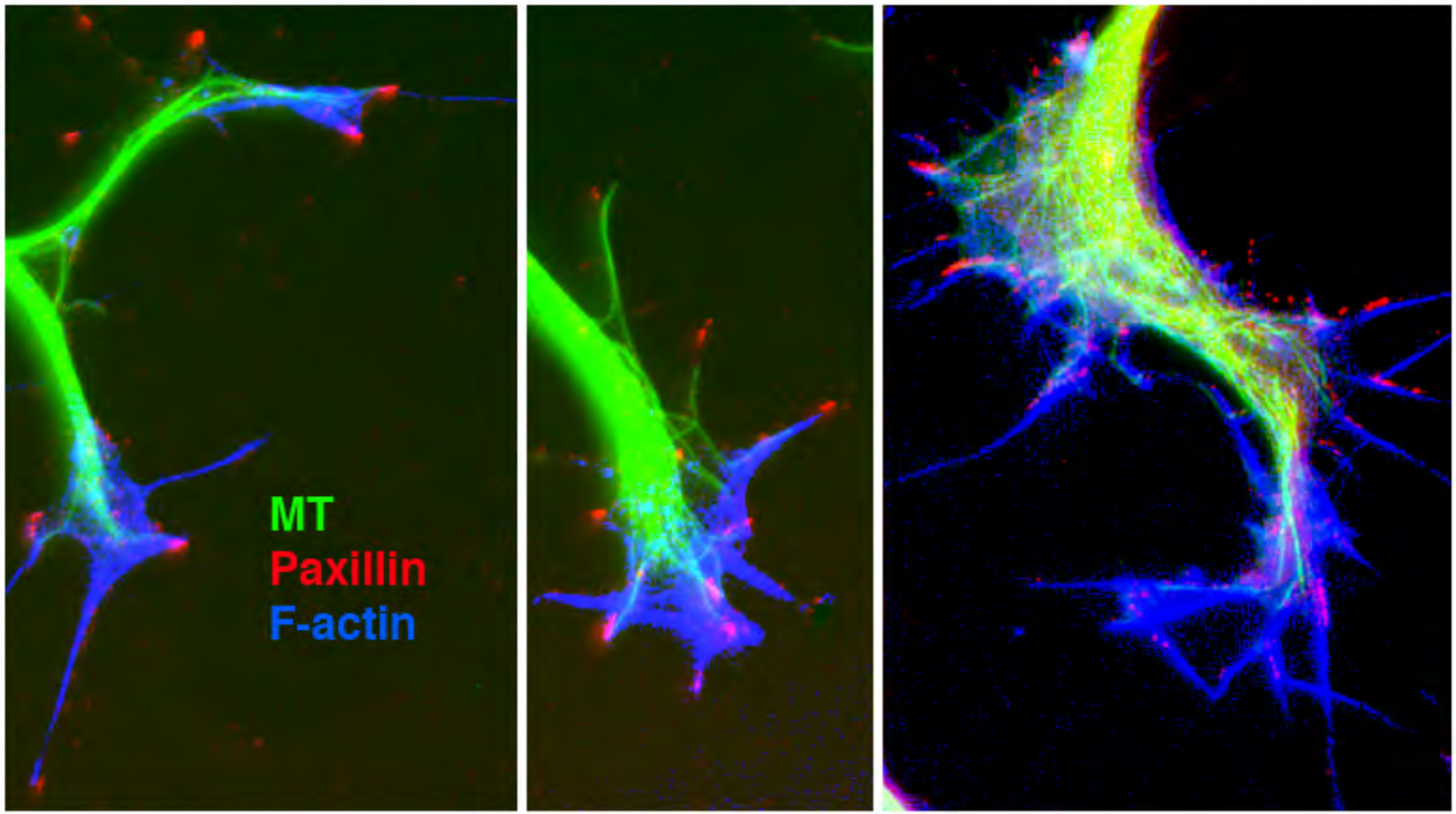


The clutch hypothesis of growth cone migration

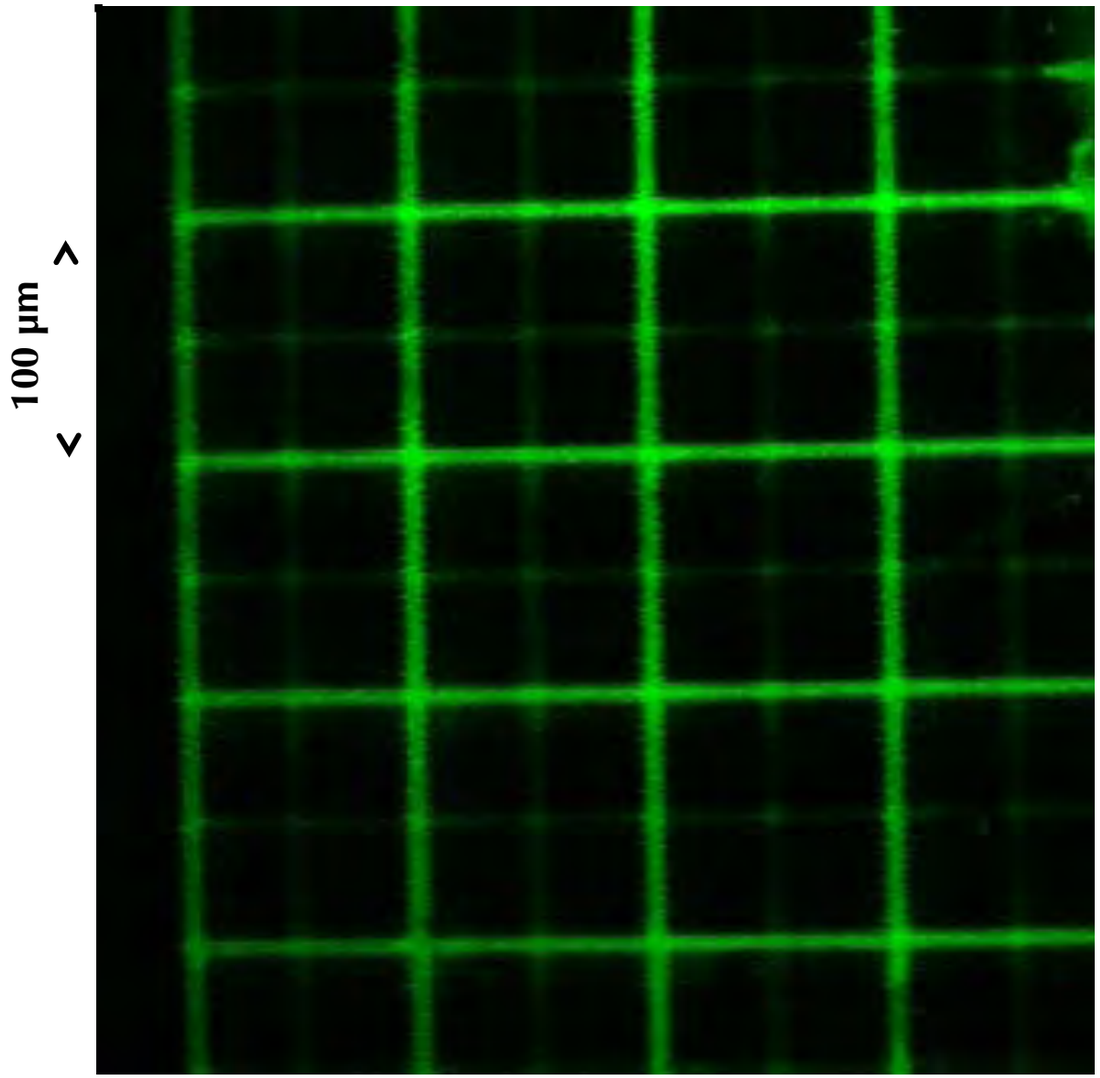
•Assembly of F-actin at the leading edge pushes filopodia and lamellipodia out.
• Retrograde flow, powered by myosin II, pulls the F-actin backward, and actin is disassembled.
•When protrusions make adhesions, a clutch is engaged to link F-actin to the stable adhesive sites. This reduces retrograde flow.
•Movements powered by motor molecules (other myosins and MT motors) and MT polymerization bring materials forward (e.g., microtubules, membrane, actin monomers) to supply the extending protrusion.



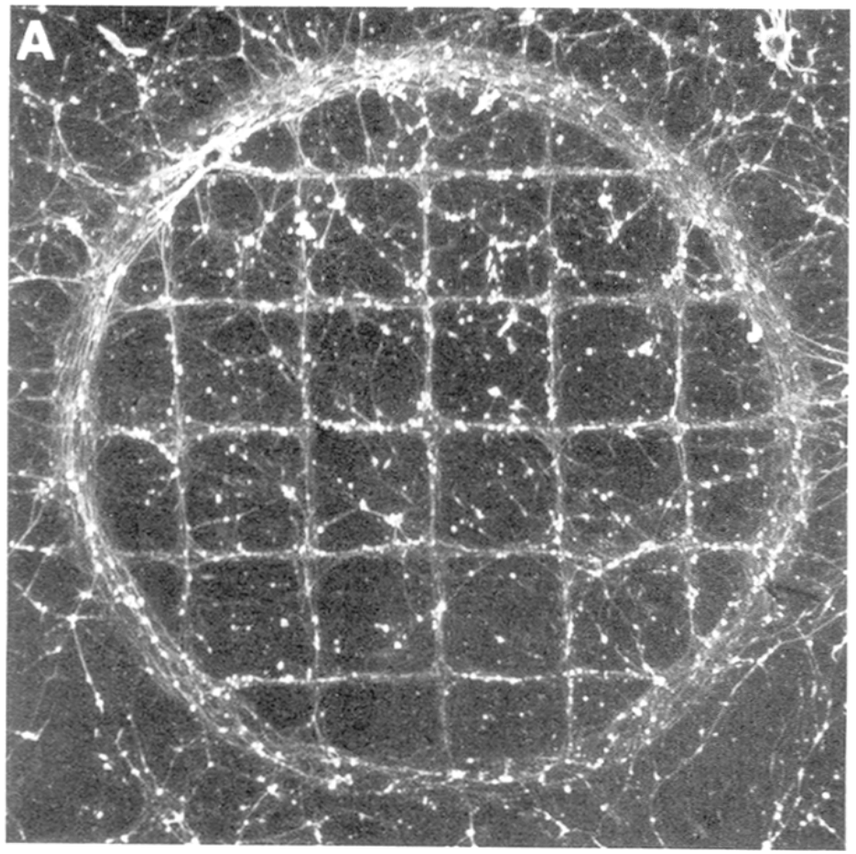
Microtubules do not extend past growth cone adhesion sites

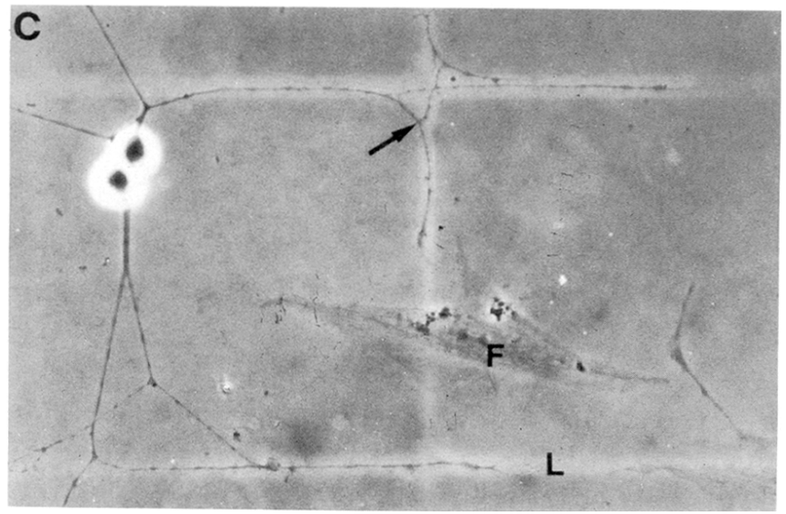


This substrate contains a grid pattern of deposition of an adhesive molecule laminin.



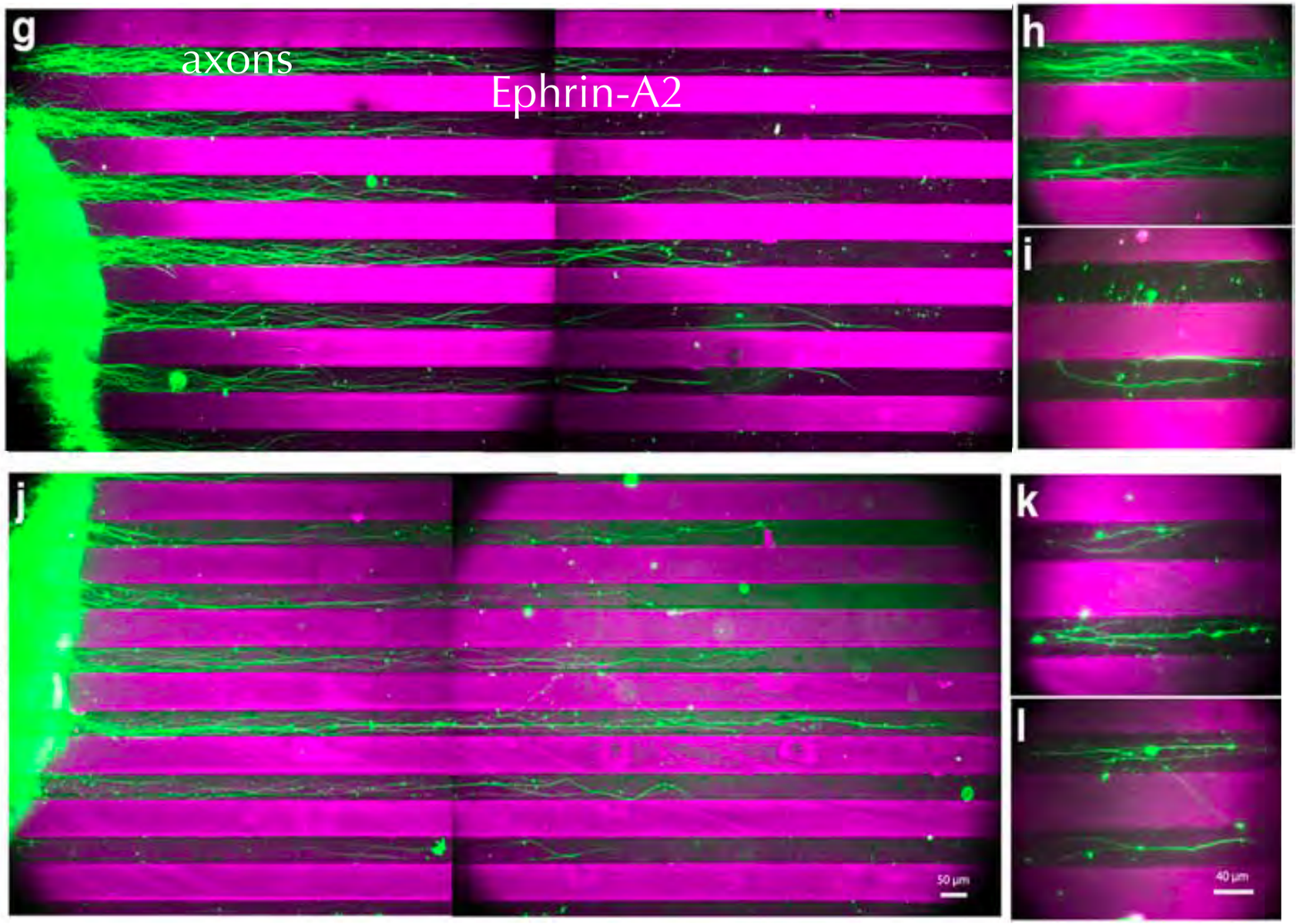
Axonal growth cones follow the patterned deposition of the adhesive protein laminin on a glass or plastic surface.





James Hammarback (1985)

Retinal axonal growth cones avoid crossing to ephrin-A2 surfaces



Guidance cues act through a series of steps to regulate growth cone behaviors

Guidance Cue

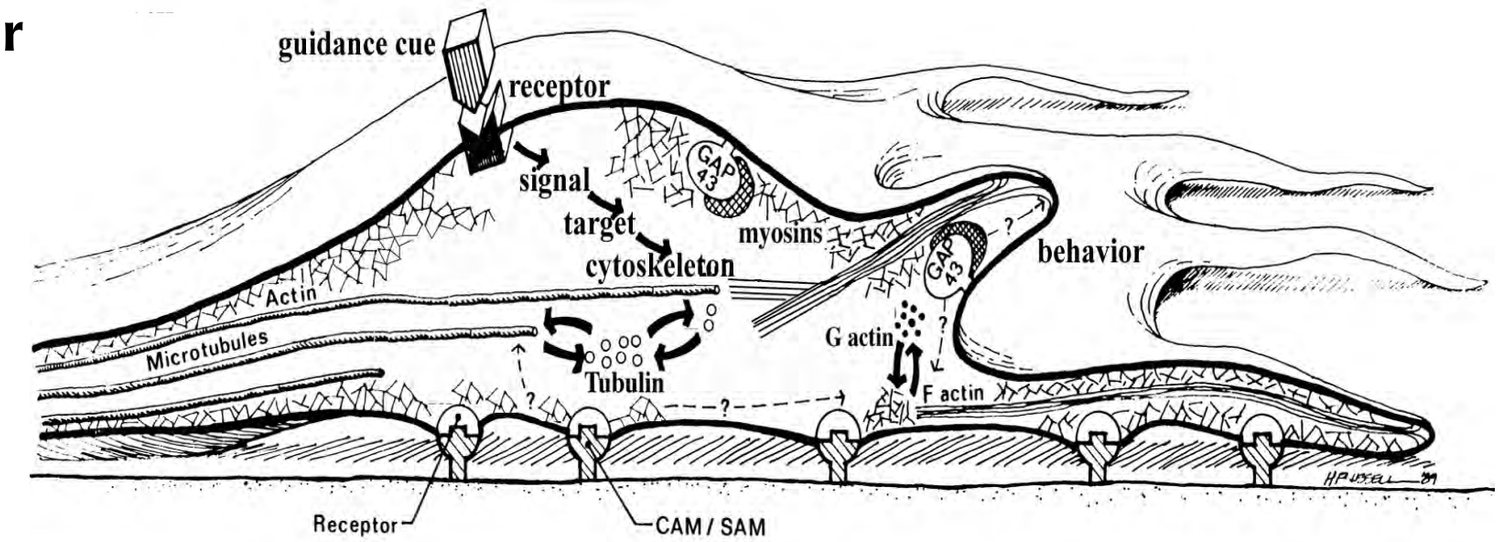
Receptor

Signal

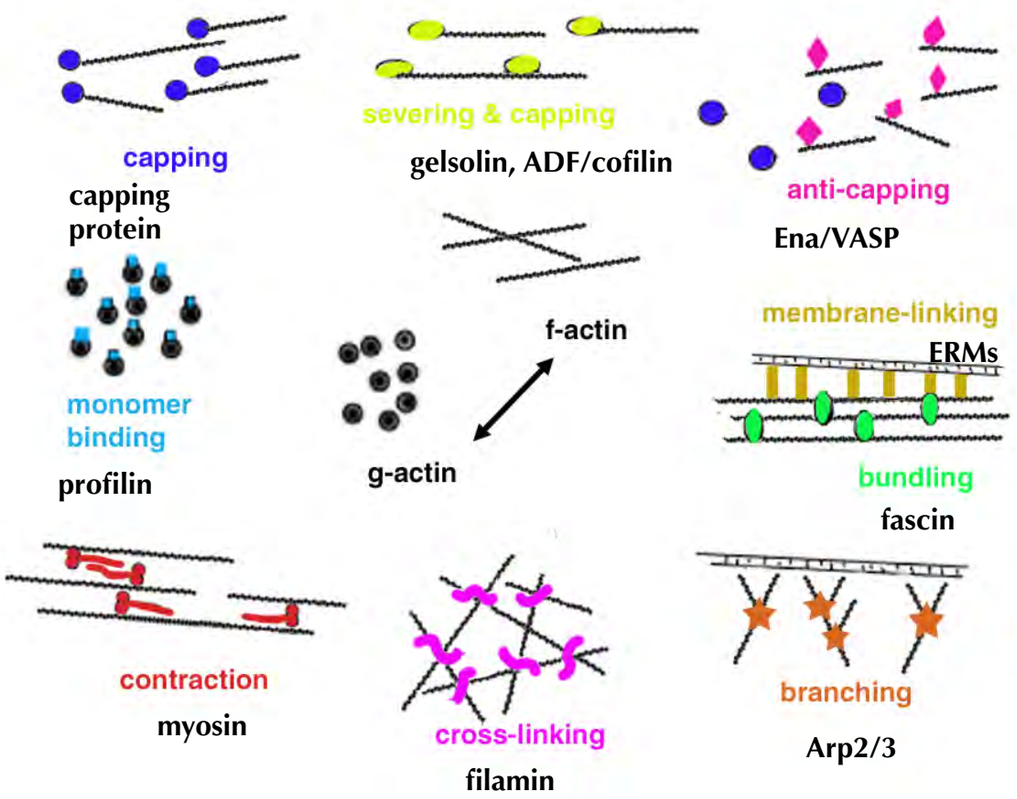
Target

Cytoskeleton

Growth Cone Behavior



Many of the targets of signaling by growth cone guidance cues are actin-binding proteins (ABPs)



- regulate actin polymerization, organization and dynamics.

- Actin organization depends on:
  - localization
  - relative concentrations
  - activity levels of actin regulatory proteins

Guidance Cue

↓

Receptor

↓

Signal

↓

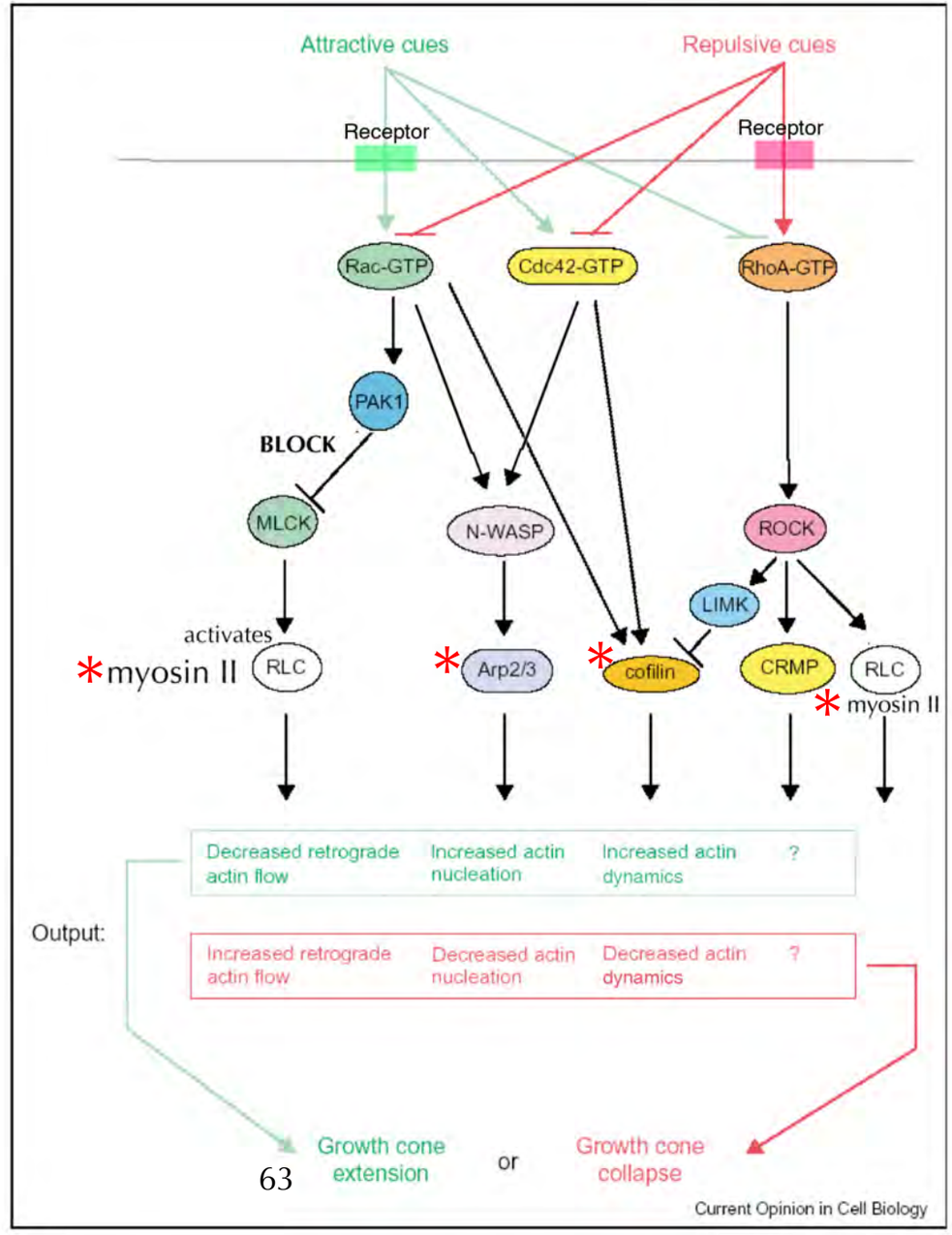
Target

↓

Cytoskeleton

↓

Growth Cone Behavior



Guidance Cue

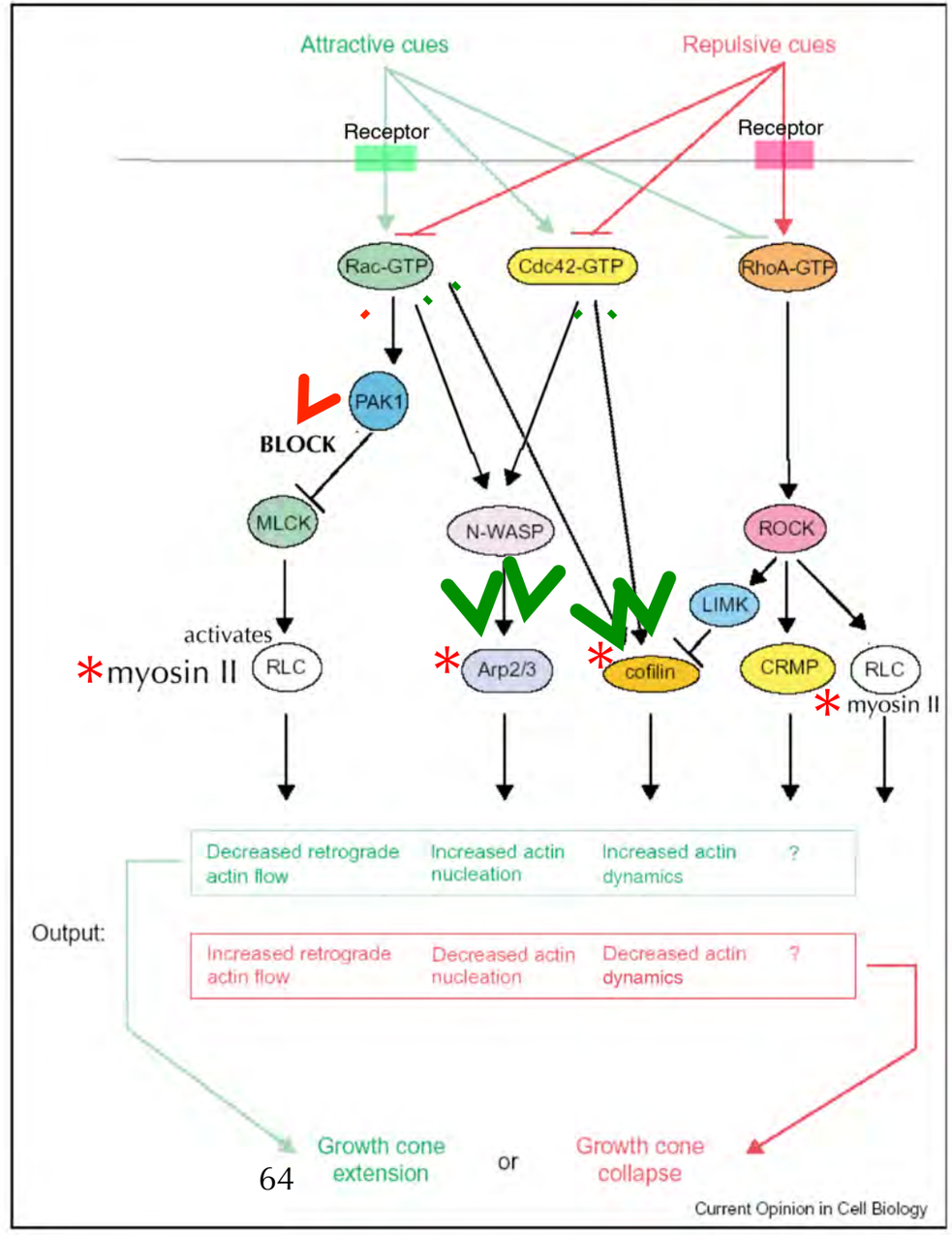
Receptor

Signal

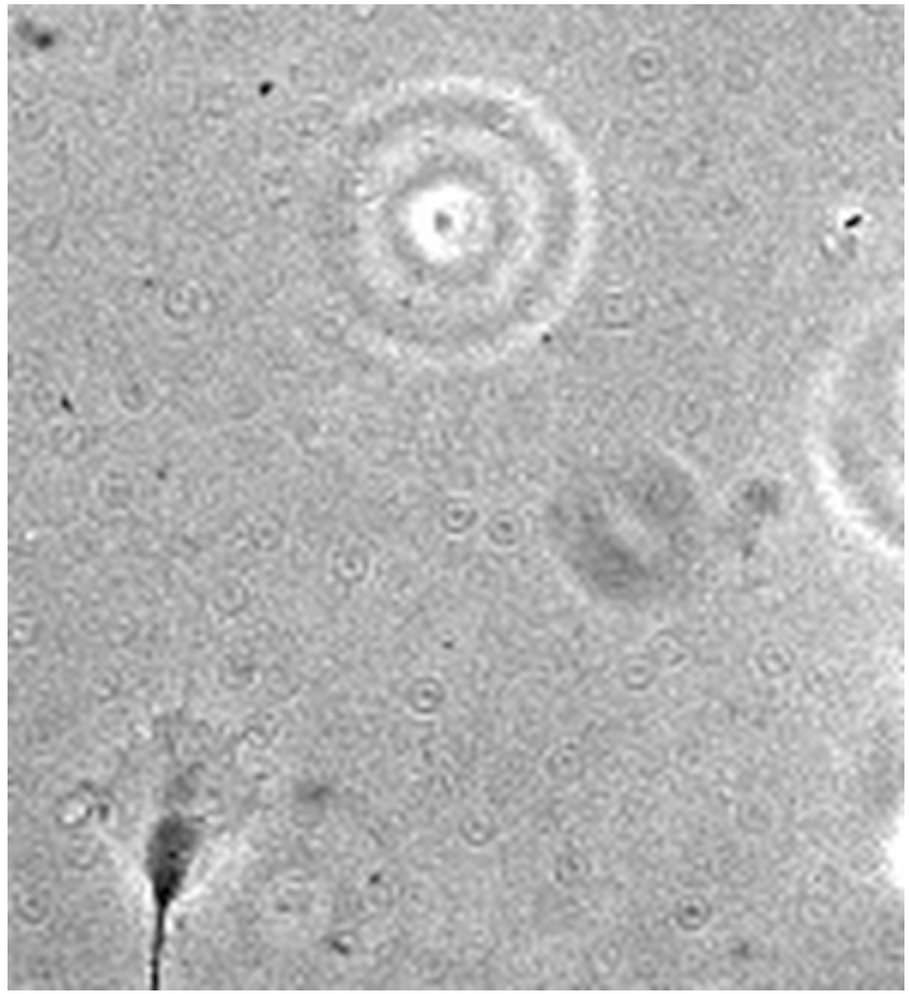
Target

Cytoskeleton

Growth Cone Behavior



Local application of the attractant netrin induces retinal growth cone turning toward the source of netrin



Guidance Cue

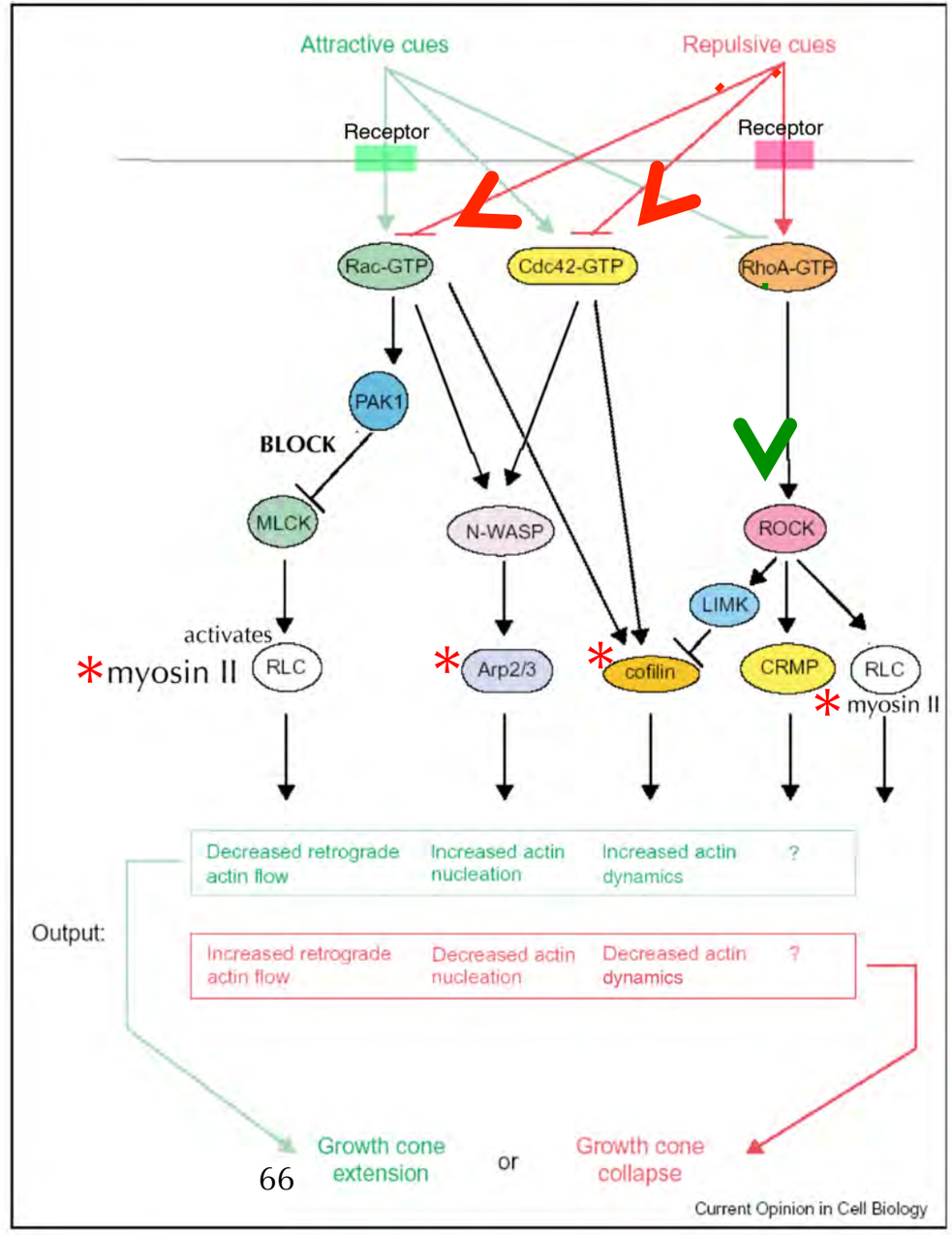
Receptor

Signal

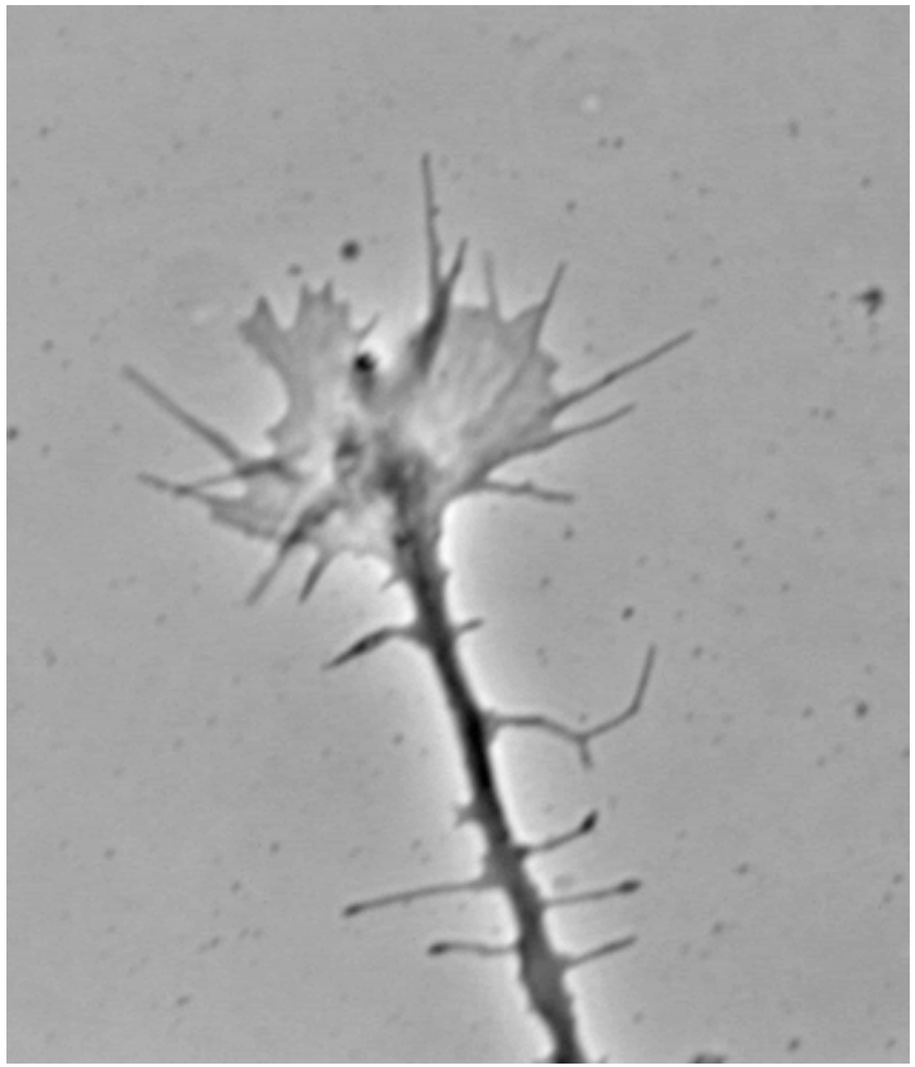
Target

Cytoskeleton

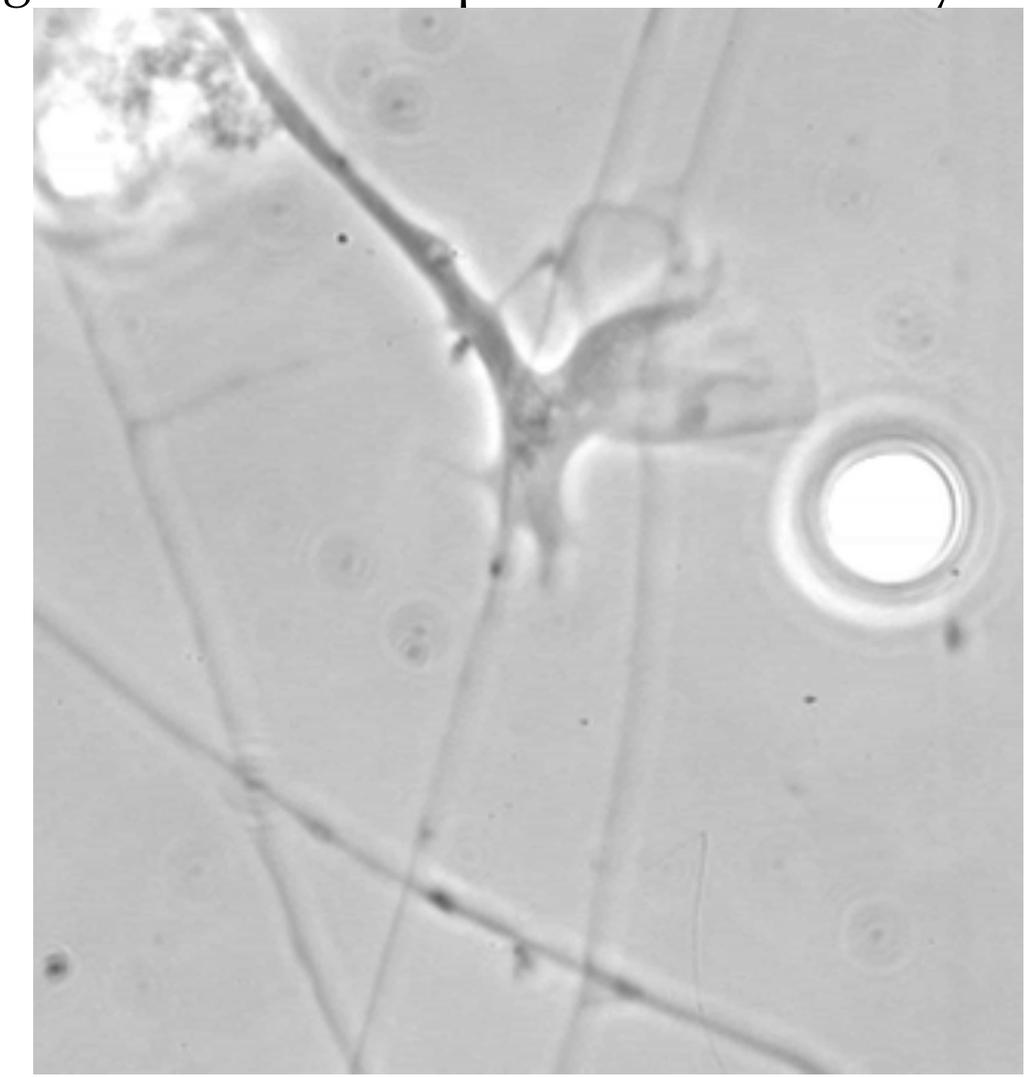
Growth Cone Behavior

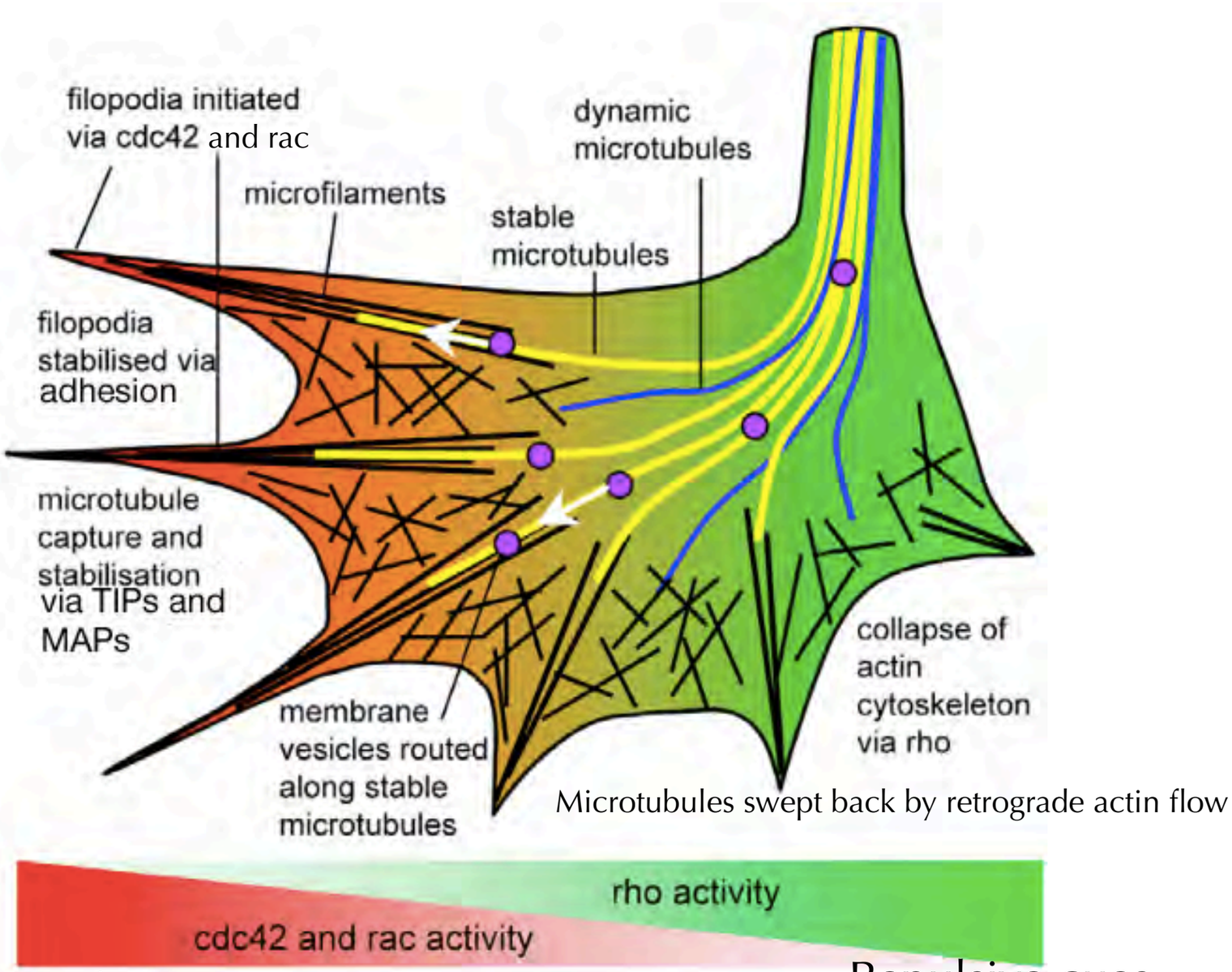


Global activation of RhoA by an repulsive guidance cue activates myosin II that creates a compressive force that collapses the growth cone and retracts the growing axon.



Local activation of RhoA by a repulsive cue on a bead induces localized growth cone collapse and a turn away from the bead.




filopodia initiated
via cdc42 and rac
microfilaments
dynamic
microtubules
stable
microtubules
filopodia
stabilised via
adhesion
microtubule
capture and
stabilisation
via TIPs and
MAPs
membrane
vesicles routed
along stable
microtubules
collapse of
actin
cytoskeleton
via rho
Microtubules swept back by retrograde actin flow
rho activity
cdc42 and rac activity
Attractive cues
Repulsive cues

Guidance Cue

↓

Receptor

↓

Signal

↓

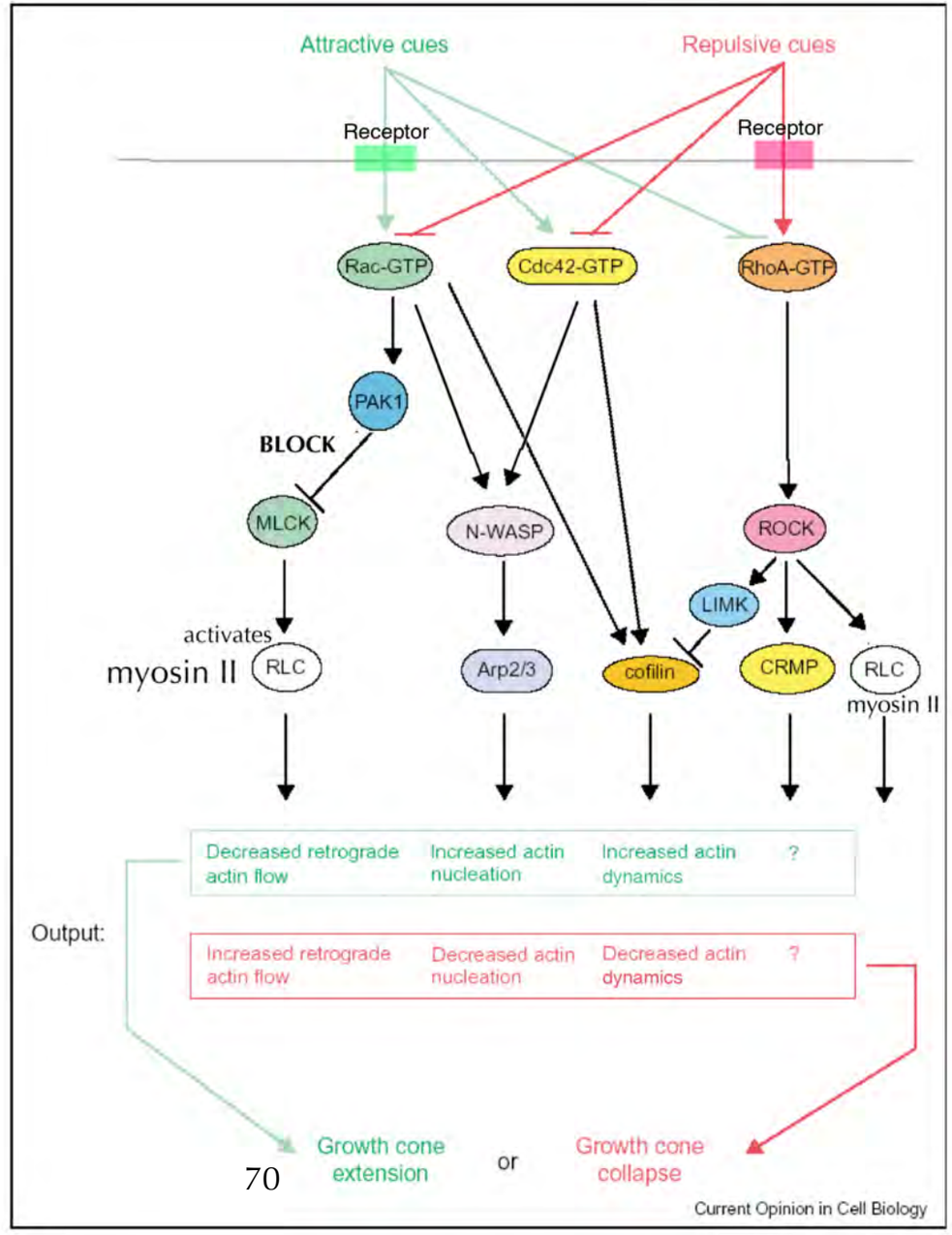
Target

↓

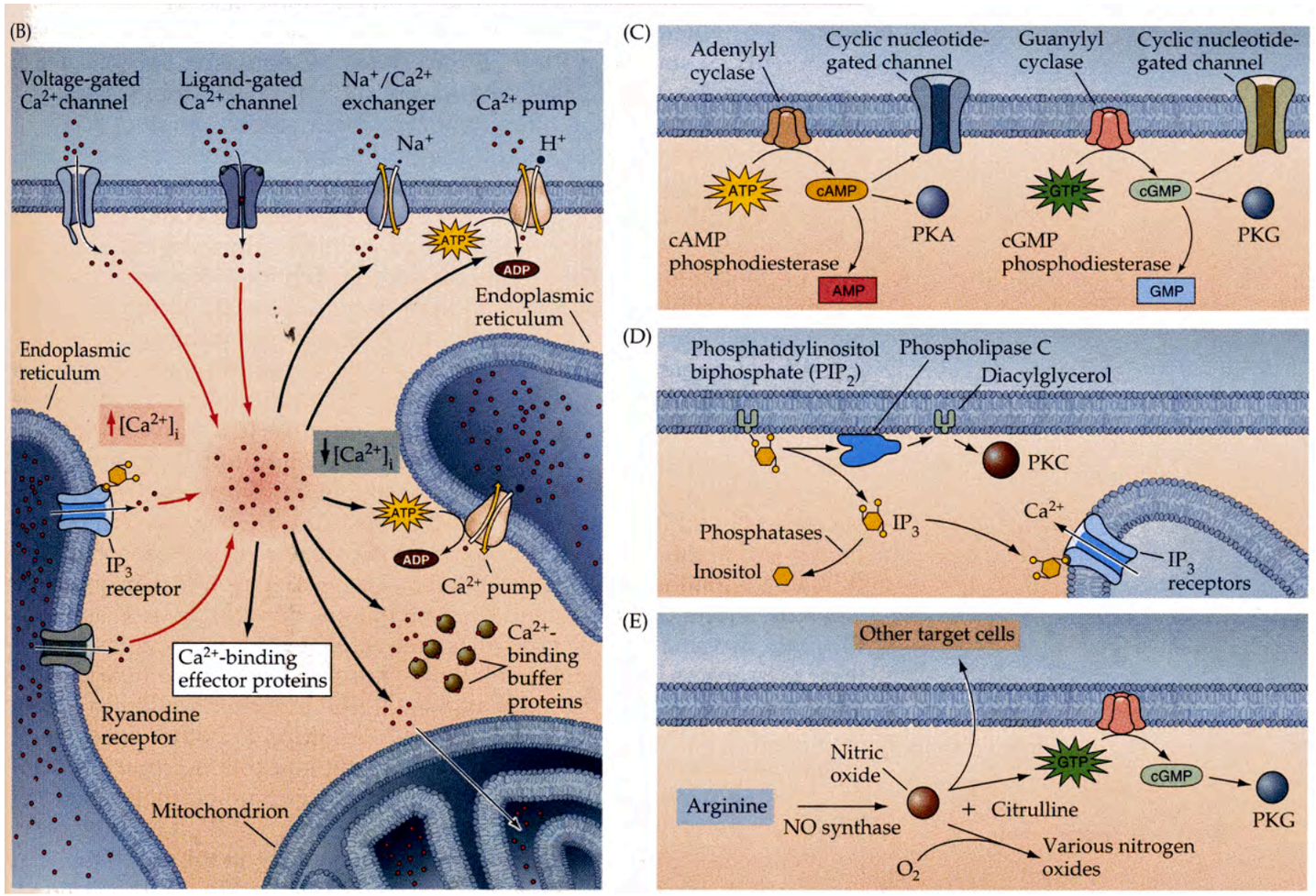
Cytoskeleton

↓

Growth Cone Behavior



Several other cytoplasmic signaling pathways modulate growth cone responses to guidance cues.



From **Neuroscience**, Purves et al. (Neuroscience 3101)

Guidance Cue

↓

Receptor

↓

Signal cAMP,cGMP
[Ca^{++}]
Nitric oxide

↓

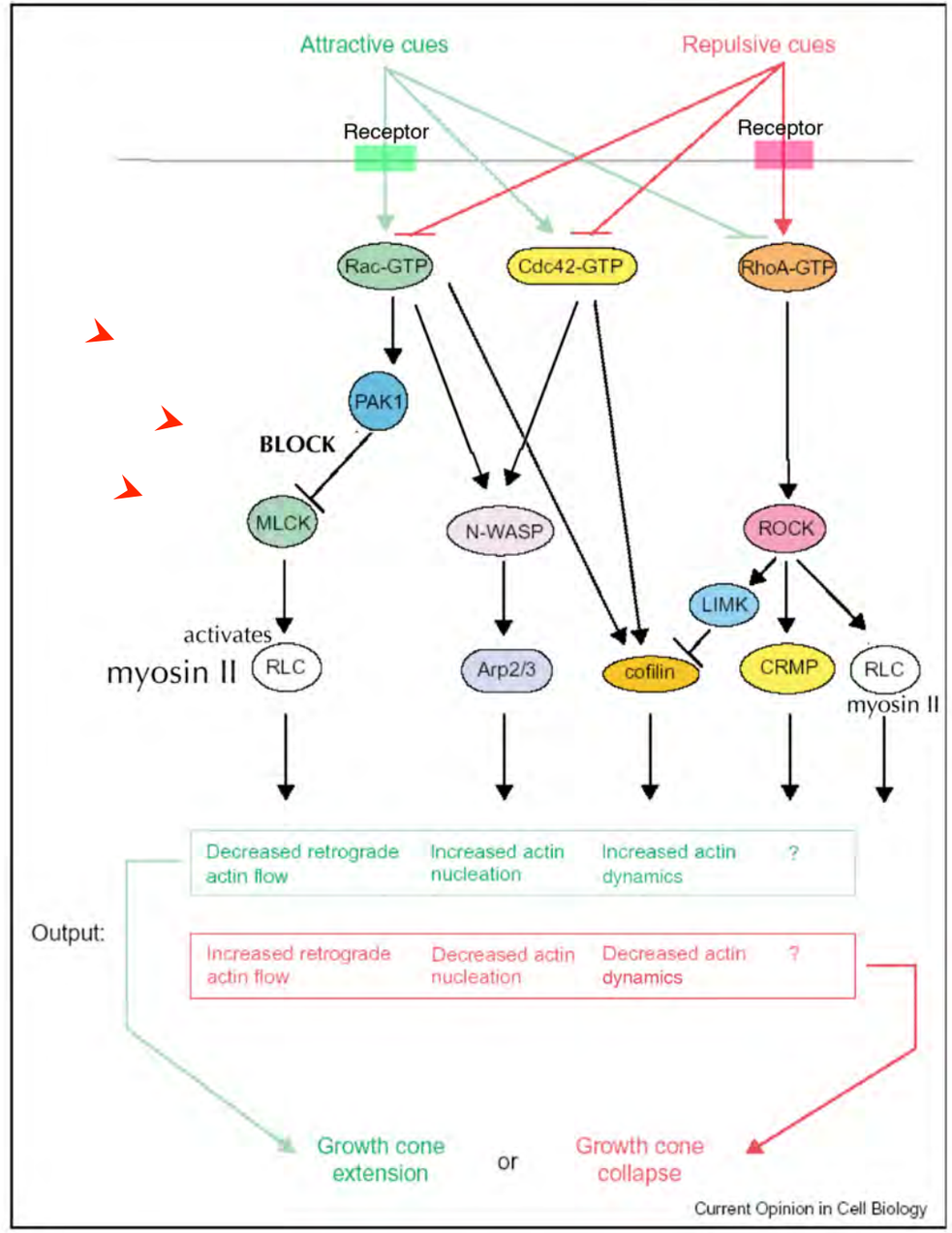
Target

↓

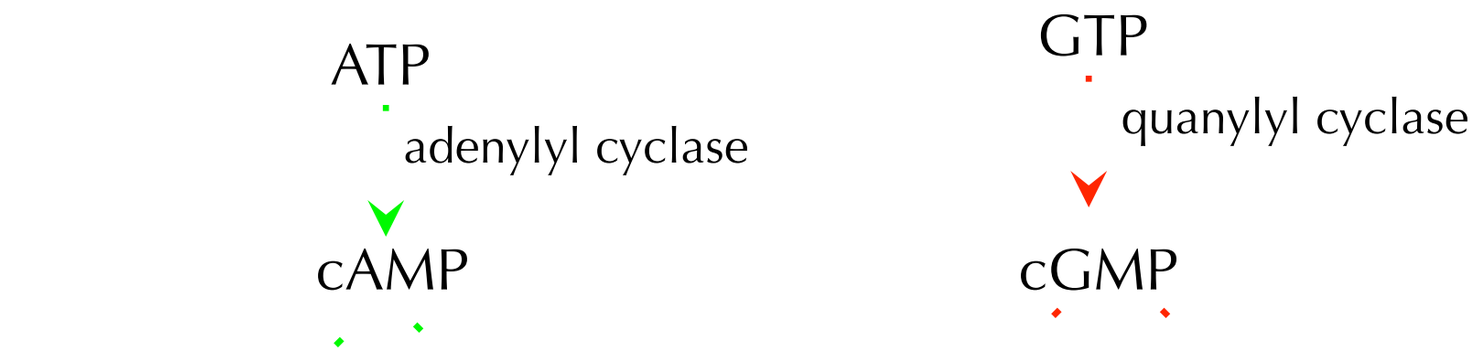
Cytoskeleton

↓

Growth Cone Behavior

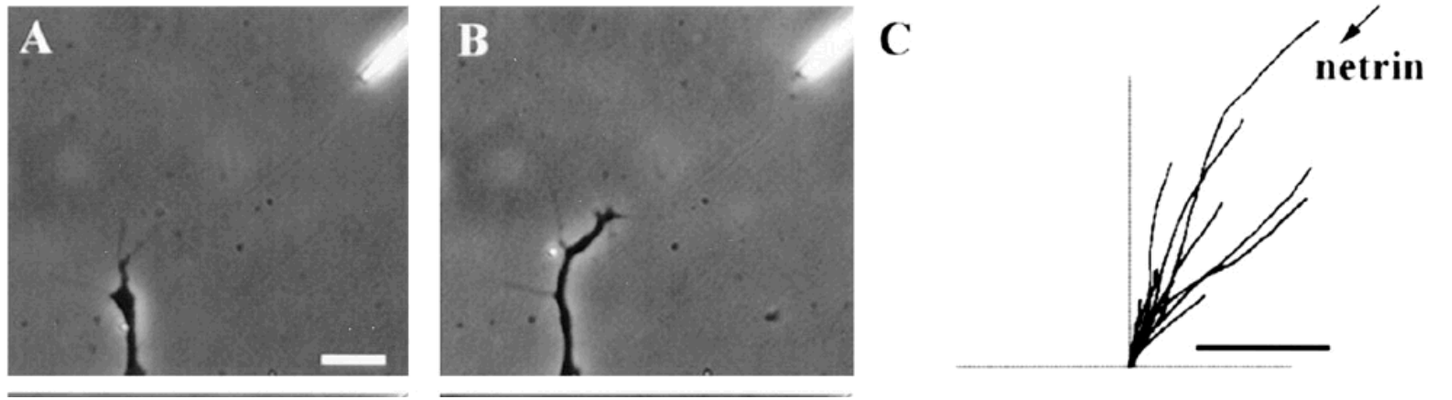


Cell Signaling

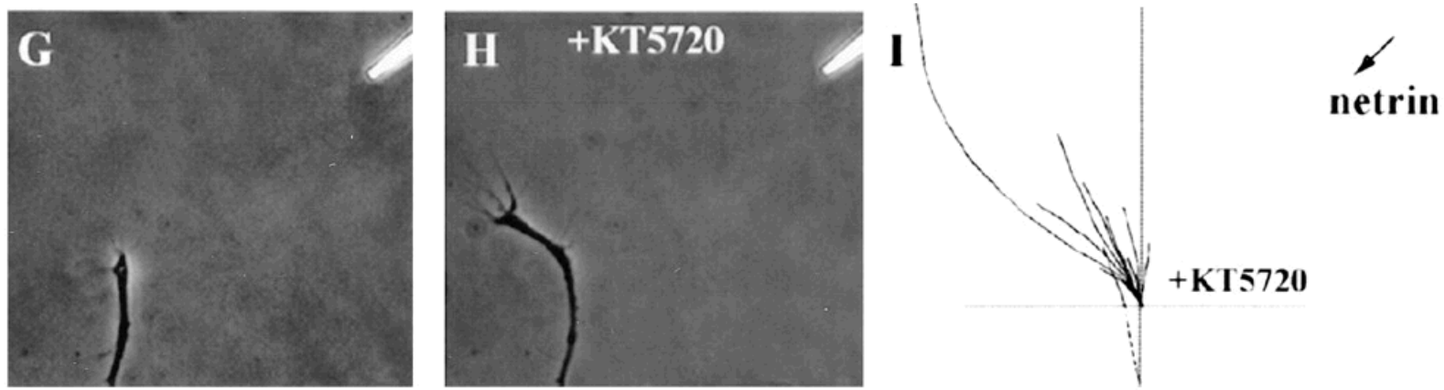


Attraction to netrin is switched to repulsion by blocking the cAMP effector PKA.

Attraction to netrin



Inhibit PKA; repulsion by netrin



Ming et al (1997), *Neuron*

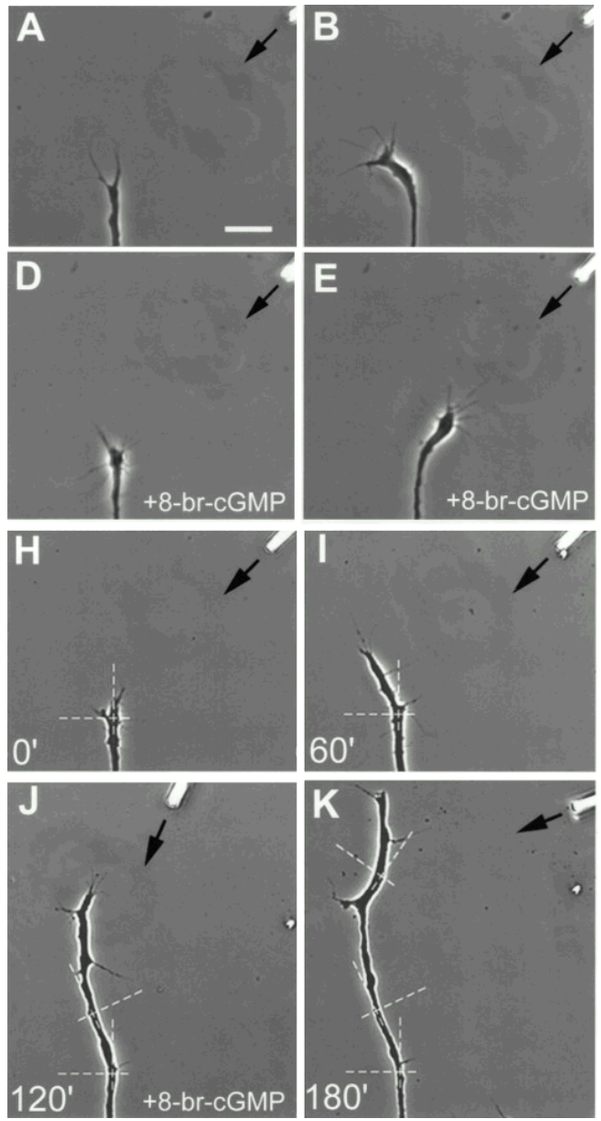
Repulsion from semaphorin 3A is switched to attraction by enhancing cGMP signaling.

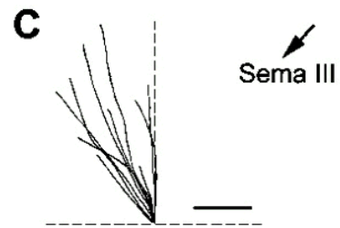
Repulsion

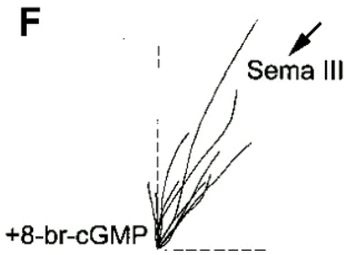
cGMP analog; attraction

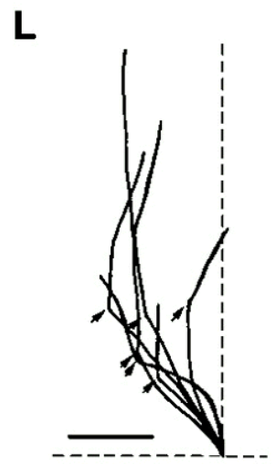
Repulsion

cGMP analog; repulsion switched to attraction









Song et al (1998), *Science*

Guidance Cue

↓

Receptor

↓

Signal cAMP,cGMP
[Ca^{++}]
Nitric oxide

↓

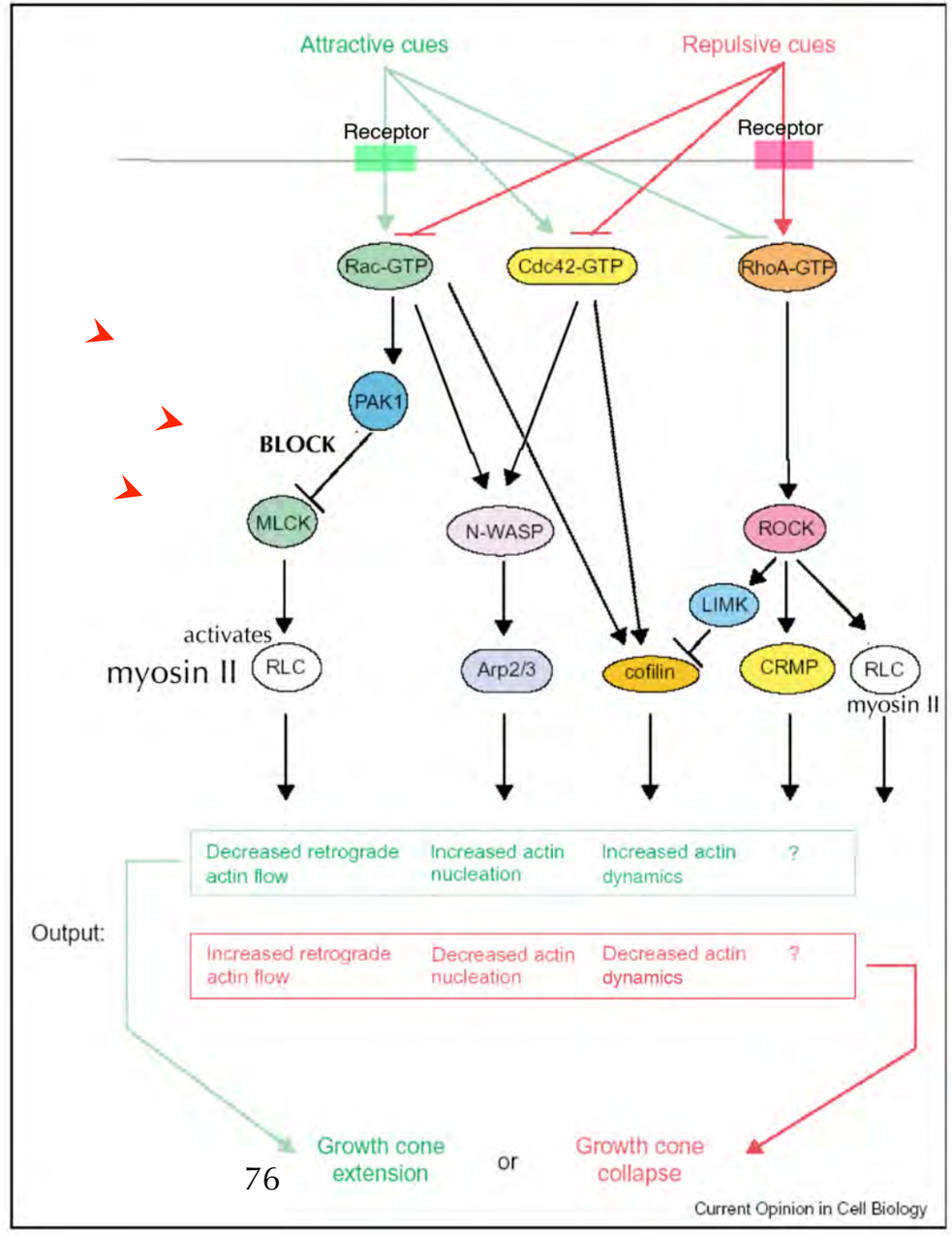
Target

↓

Cytoskeleton

↓

Growth Cone Behavior

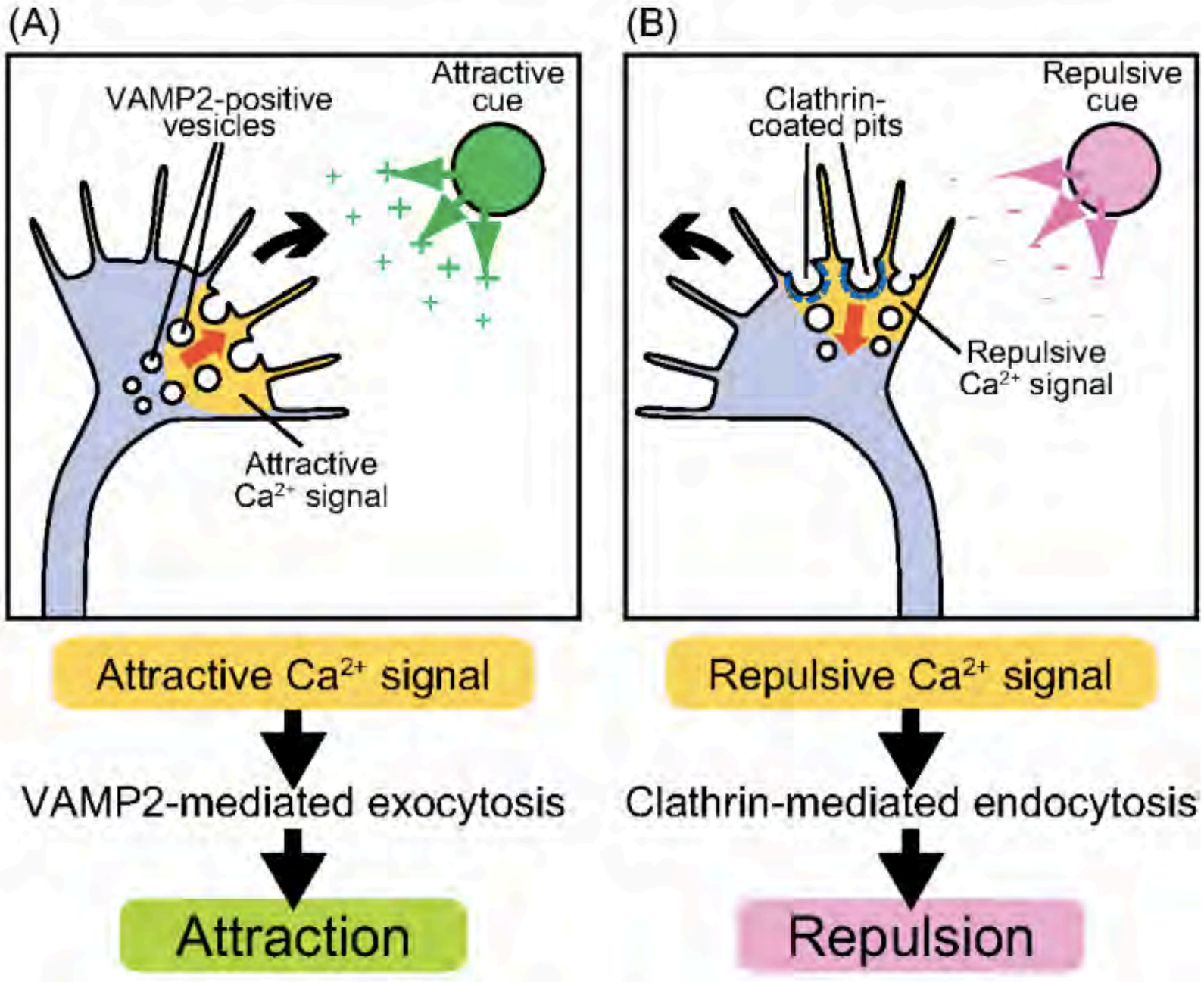


Cytoplasmic [Ca^{++}] is an important growth cone regulatory factor.

- Ca^{++} concentration, $[Ca^{++}]_i$ in the cytoplasm, is an important second messenger.
- The functions of many cytoplasmic proteins are switched on and off by binding Ca^{++} as an allosteric factor.
- Cytoplasmic [Ca^{++}] is regulated by ion channels and pumps located in the plasma membrane, ER and other internal membrane systems.

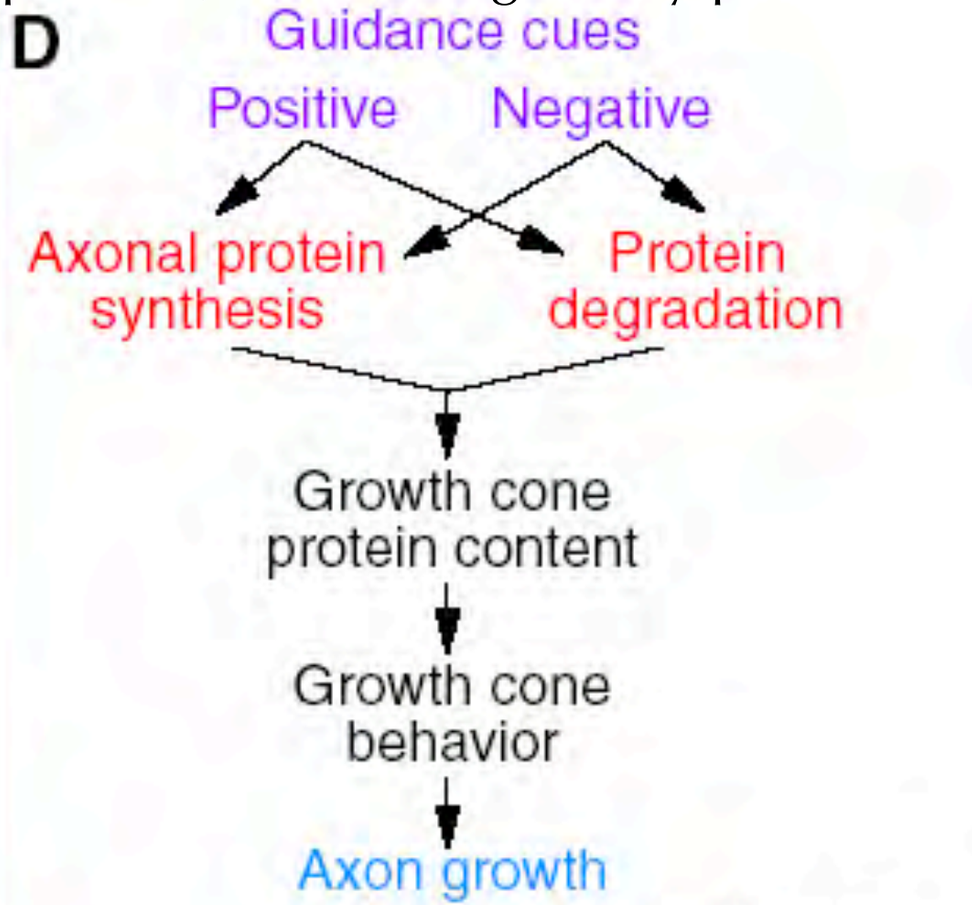
Growth cone proteins regulated by [Ca^{++}]

calmodulin (regulatory protein)
CAM kinase
PKC (kinase)
calcineurin (phosphatase)
gelsolin (breaks actin filaments)
α–actinin (bundles actin filaments)
myosin II (motor protein)

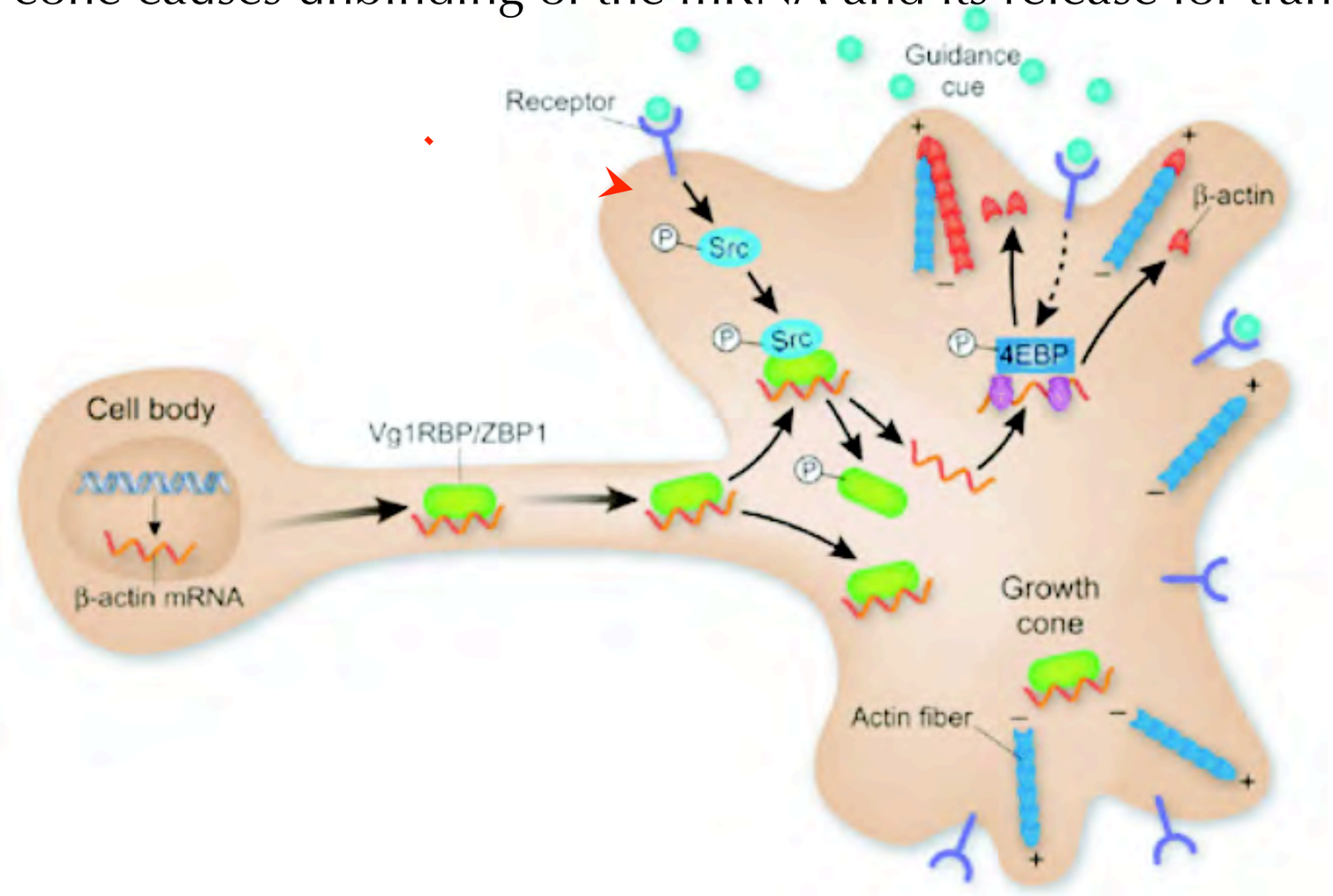

(A)
VAMP2-positive vesicles
Attractive cue
Attractive Ca2+ signal
Attractive Ca2+ signal
VAMP2-mediated exocytosis
Attraction
(B)
Clathrin-coated pits
Repulsive cue
Repulsive Ca2+ signal
Repulsive Ca2+ signal
Clathrin-mediated endocytosis
Repulsion

•Up to now we have discussed growth cone guidance in terms of regulating the activities of growth cone proteins.

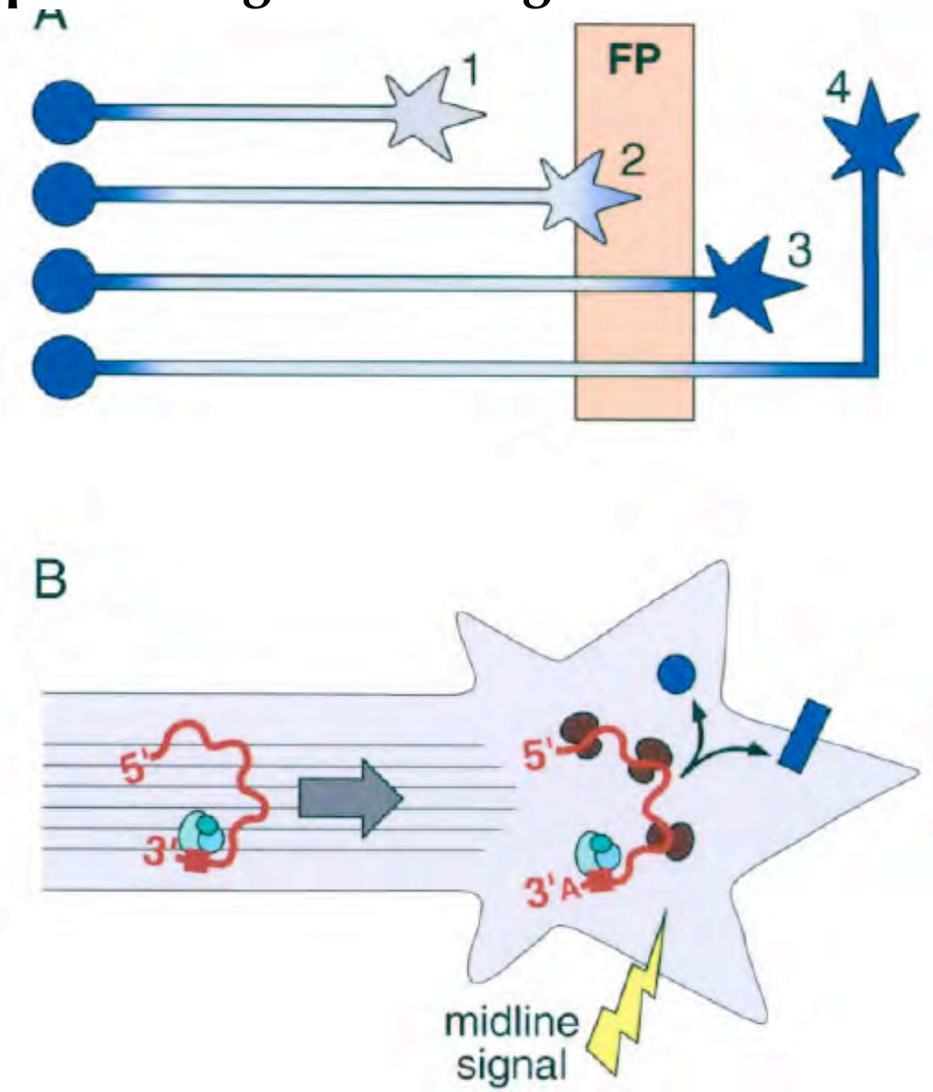
•However, growth cone behaviors are also regulated through the the local synthesis and degradation of guidance cue receptors, signaling components, or actin regulatory proteins.



Certain mRNAs are transported to growth cones and axon terminals in complexes with mRNA-binding proteins that recognize a 3' UTR, called the zipcode. Signaling in the growth cone causes unbinding of the mRNA and its release for translation.



When certain growth cones cross the CNS midline, a midline signal induces translation of local mRNA in growth cones and insertion of receptors for guidance ligands.



Guidance Cue

↓

Receptor

↓

Signal — cAMP,cGMP
[Ca^{++}]
Nitric oxide

↓

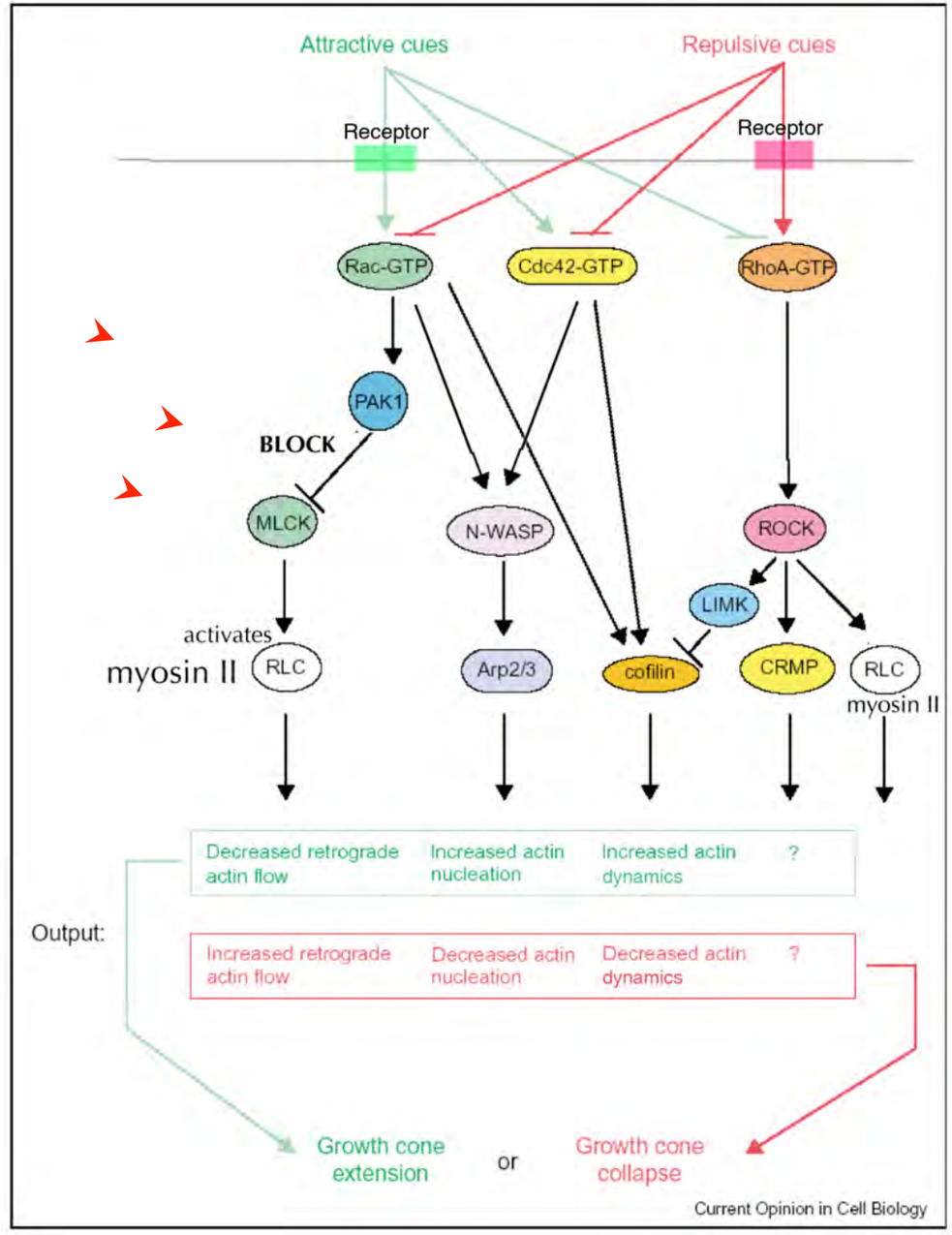
Target — Protein synthesis, degradation

↓

Cytoskeleton

↓

Growth Cone Behavior



Growth cones must integrate signaling simultaneously triggered by multiple guidance cues.

Guidance Cue

Receptor

Signal

Target

Cytoskeleton

Growth Cone Behavior