Axon guidance I

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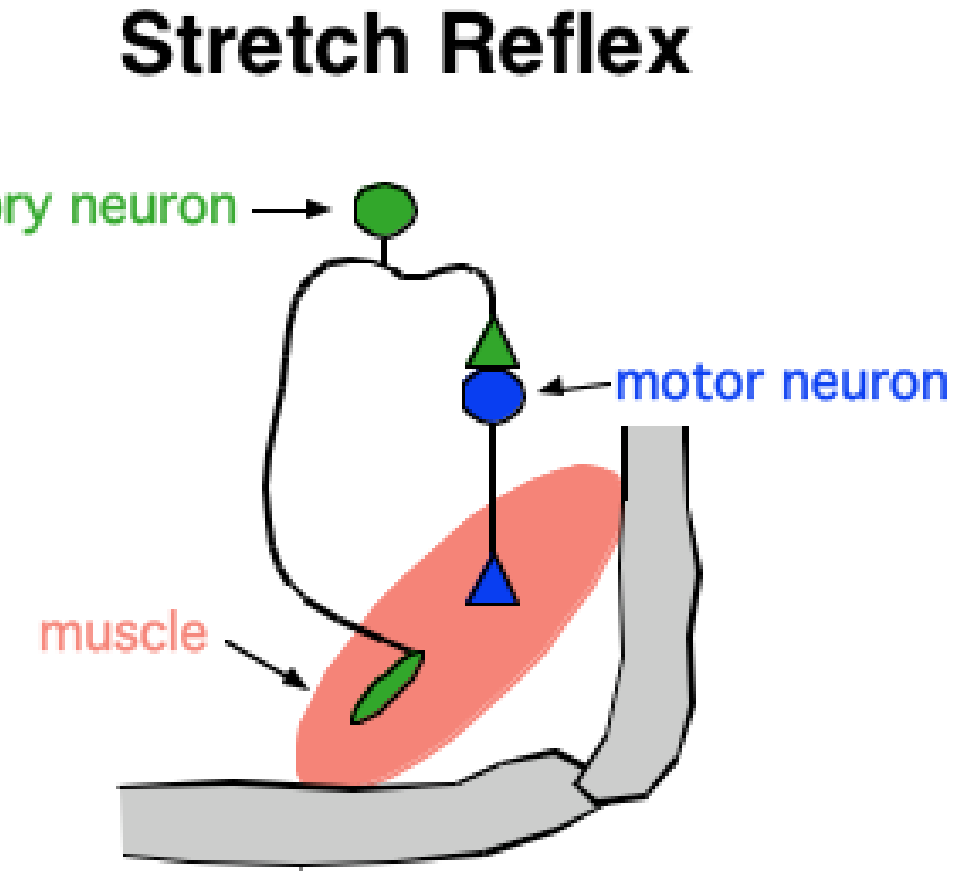
7.68/9.013

Neuronal Wiring:
Functional Framework of the Nervous System

Stretch reflex circuit

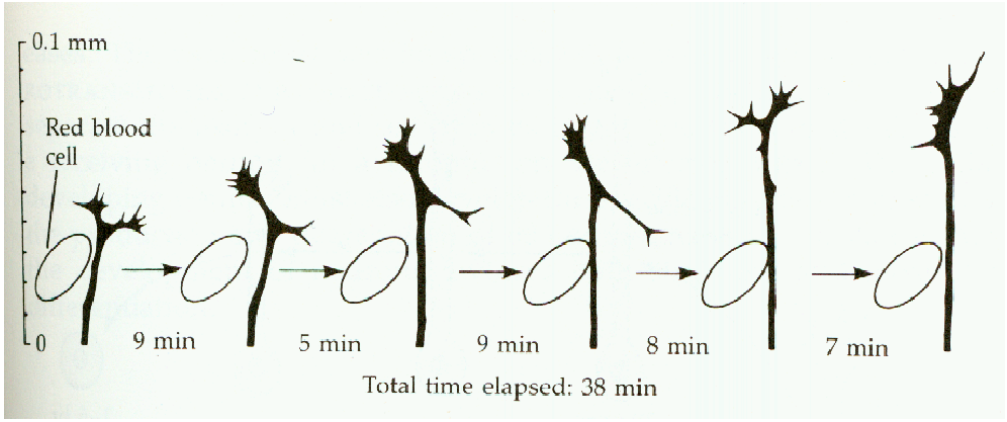


Early theories of axonogenesis

- Schwann: many neurons link to form a chain
- Hensen: axon forms around preexisitng threads between cells
- Wilhelm His (1886) and Santiago Ramon y Cajal (1890): Proposed that axon is an outgrowth from a neuron

Axon outgrowth

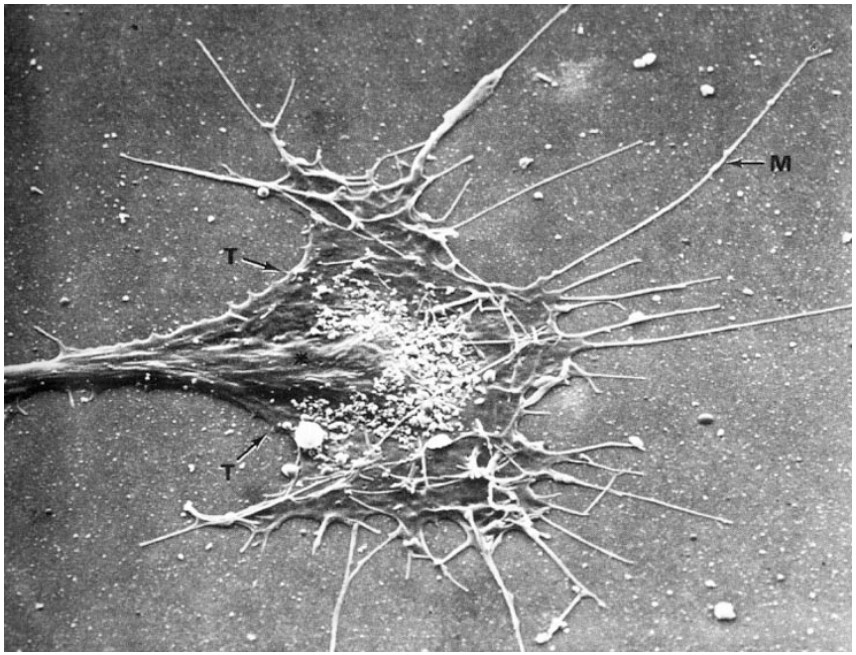
- **Ross Harrison (1907):Invented tissue culture to demonstrate axon extension**
  - **Isolated piece of neural tube from tadpole**
  - **Placed neuroblasts in drop of frog lymph on coverslip inverted over depression slide**
  - **Watched axons emerge from differentiating neurons in his "hanging drop" prep at 56 microns/hour**



Adapted from Harrison (1908)

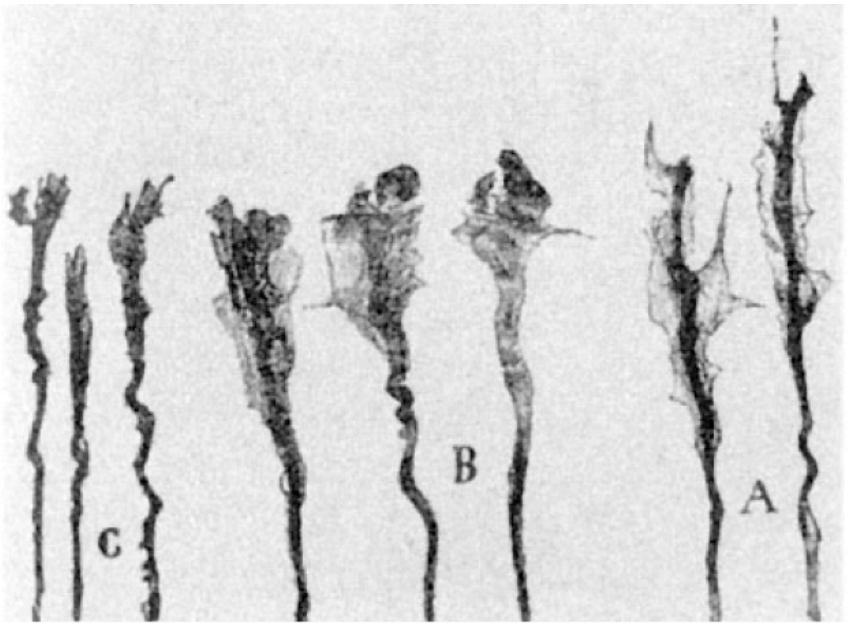
The growth cone

- **At leading edge of the axon (and dendrite) is a motile structure where much of the control of axon navigation takes place: the growth cone**



Growth cones are highly dynamic

- **Growth cones crawl over a variety of surfaces to reach their targets and have a wide variety of shapes *in vivo***

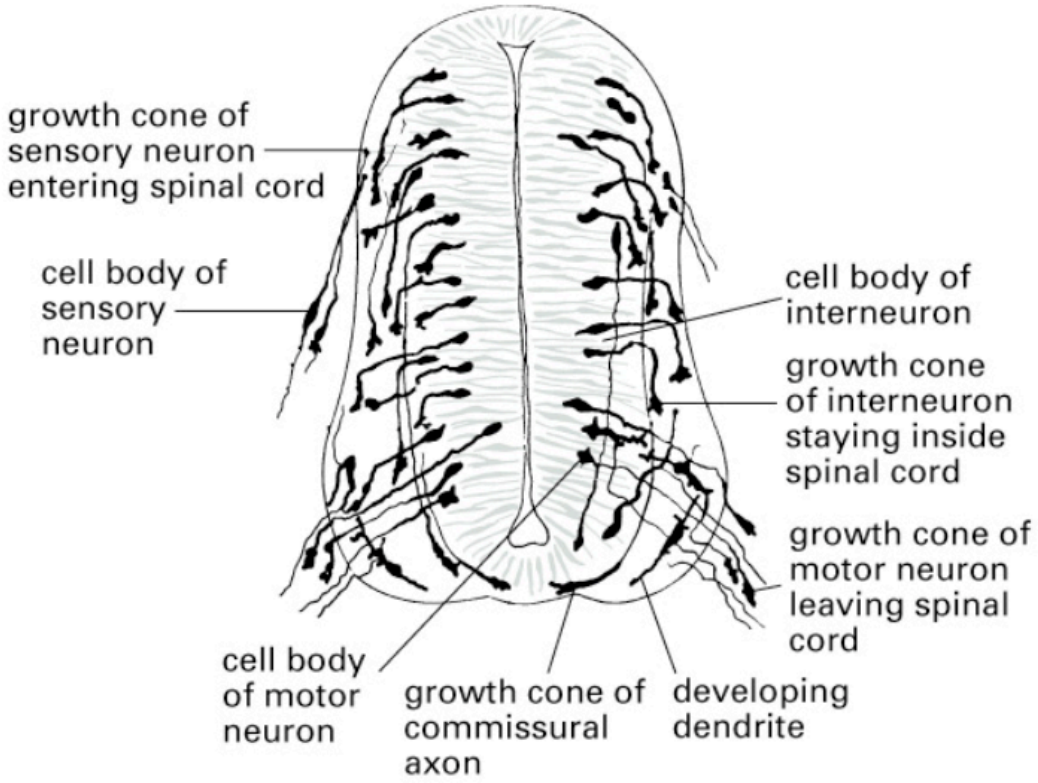


Ramon y Cajal 1890

Movies of axon guidance in vivo

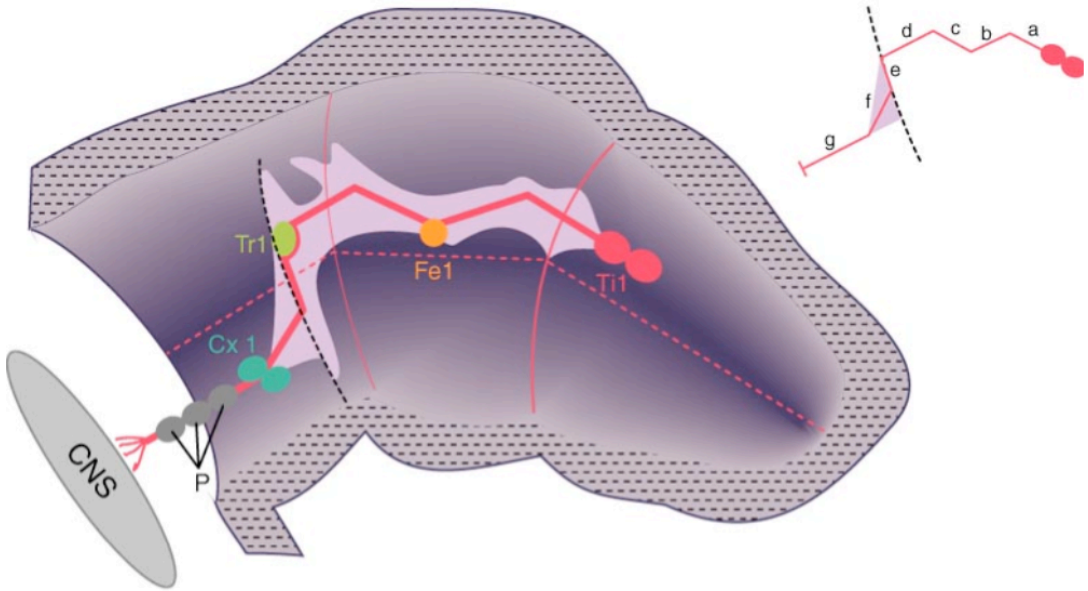
- **Xenopus spinal cord**
  - **http://gomez.anatomy.wisc.edu/Lab%20Page%20folder/Lab%20Page/SpinalCord.html**
    - » **5 min/frame; 5 hours;**
- **Xenopus visual system**
  - **http://www.anat.cam.ac.uk/pages/staff/academic/holt/large.mov**
    - » **3 min/frame; 6 hours**

Neurons show cell-type specific axon projection patterns



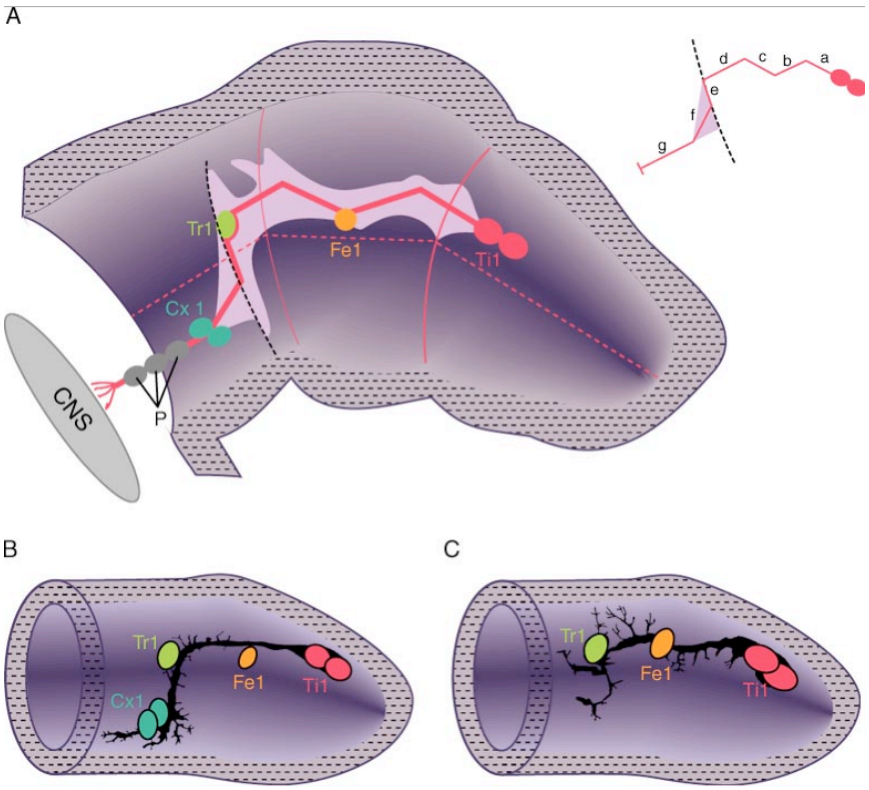
An axon's complex journey can be broken into discrete segments

- Axons navigate using a series of intermediate targets
  - Example: Ti1 neuron in grasshopper limb bud



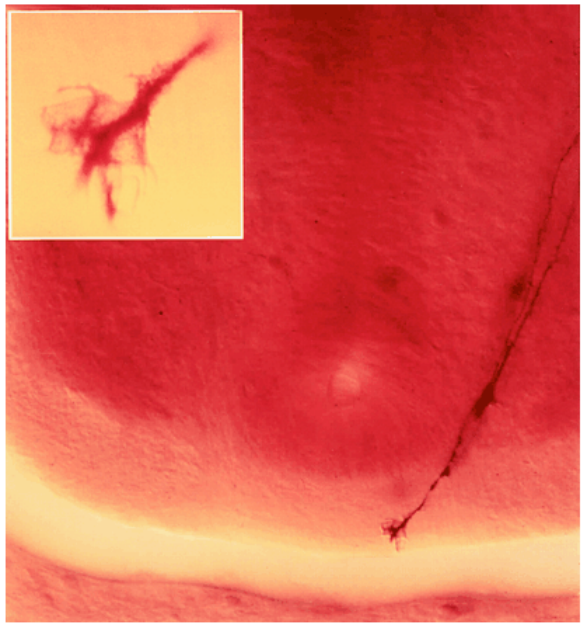
Intermediate targets play essential role in navigation

- **Ablate Cx1 cell -- axon halts**



How are axons guided?

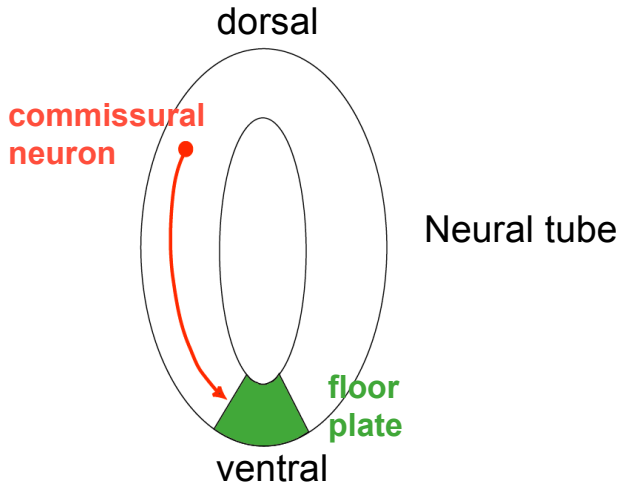
- **Ramon y Cajal (1892): Chemotropism**
  - **Axons guided by diffusible cues from target cells : based on anatomical observation**



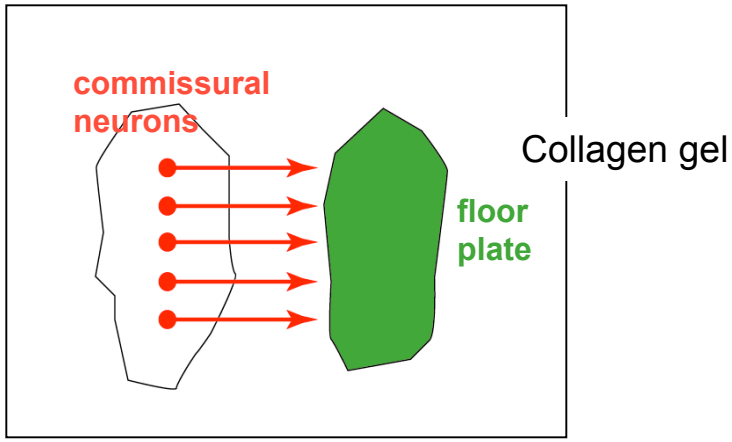
Cajal (1890), day 4 chick spinal cord

Are axons guided by chemotropism?

- **YES: 1980's: Target tissues shown to attract appropriate axons at a distance *in vitro***
- **Example: vertebrate spinal cord**

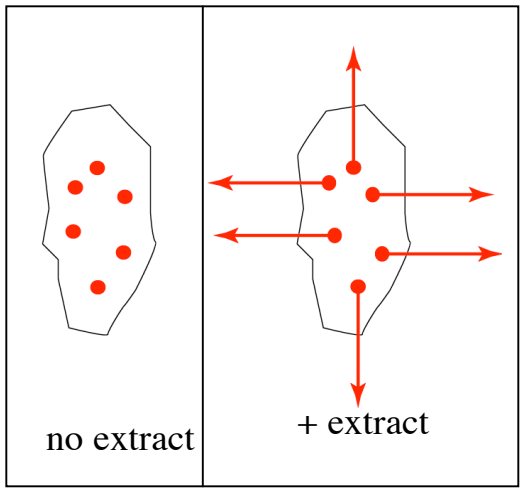


- **Floor plate explant attracts commissural axons**



What is the floor plate chemoattractant?

- Marc Tessier-Lavigne's lab (1994)
  - 20,000 chick embryonic brains
  - Fractionated protein extracts
  - Followed outgrowth promoting activity
  - Isolated two major proteins
  - Sequenced the proteins
  - Cloned two related proteins: netrin-1 and netrin-2



Netrins

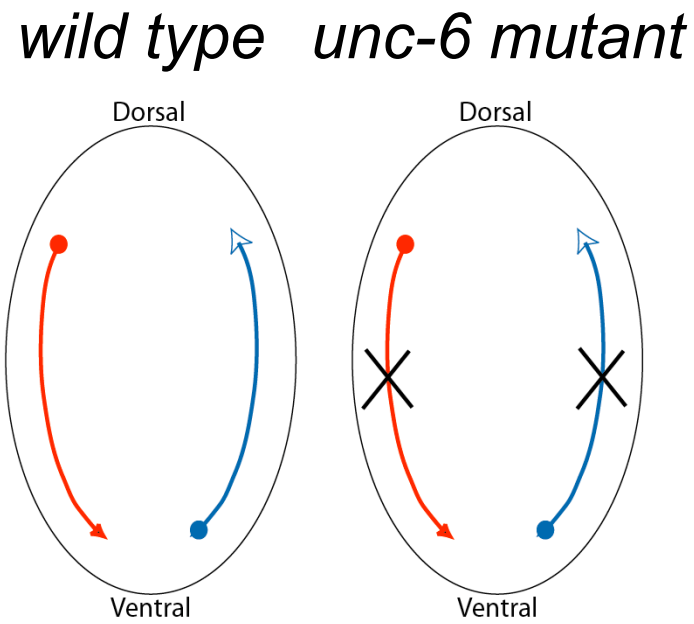
- Secreted proteins
- Related to a portion of the extracellular matrix protein laminin
- Associated with cell surfaces and the extracellular matrix

Netrin-1

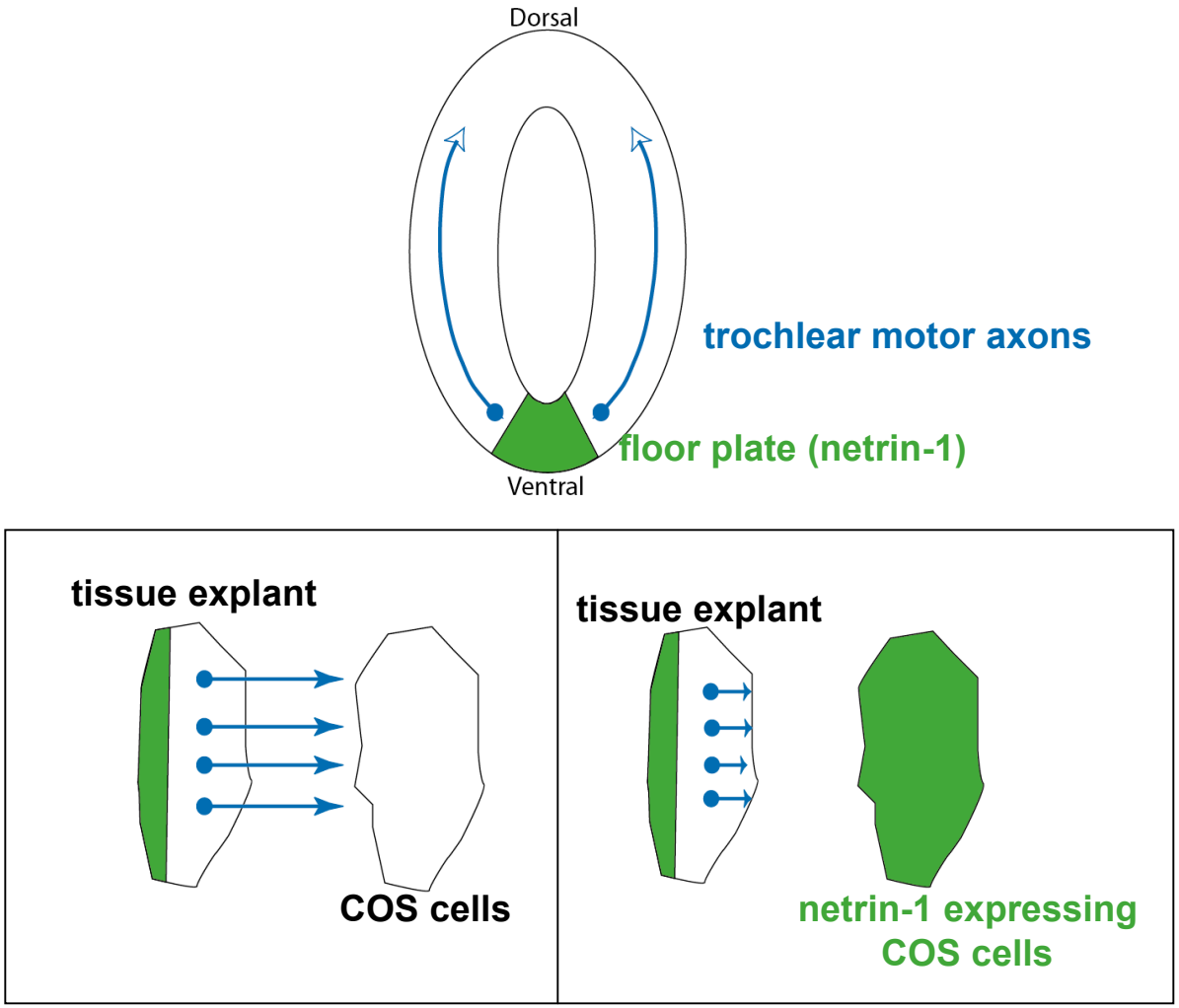
- Netrin-1 is an important floor plate chemoattractant
  - Expressed by floor plate cells
  - Netrin-1 mutant mice have commissural axon guidance defects
- And….

Netrin-1 is 50% identical to C. elegans UNC-6

- **Unc-6: found as a mutation that affects the nematode's movement (uncoordinated)**
- ***unc-6* mutations disrupt axon guidance along DV axis**
- **Netrin-1/unc-6: evolutionarily conserved midline chemotropic factor**
- **Attractant and repellent?**

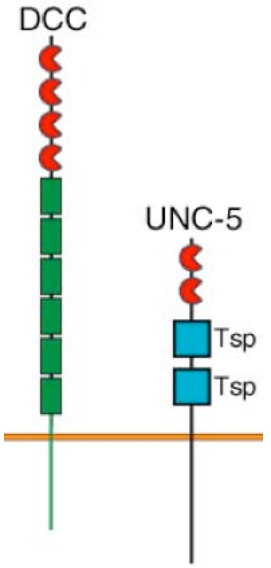


Netrin-1 can also function as a chemorepellent



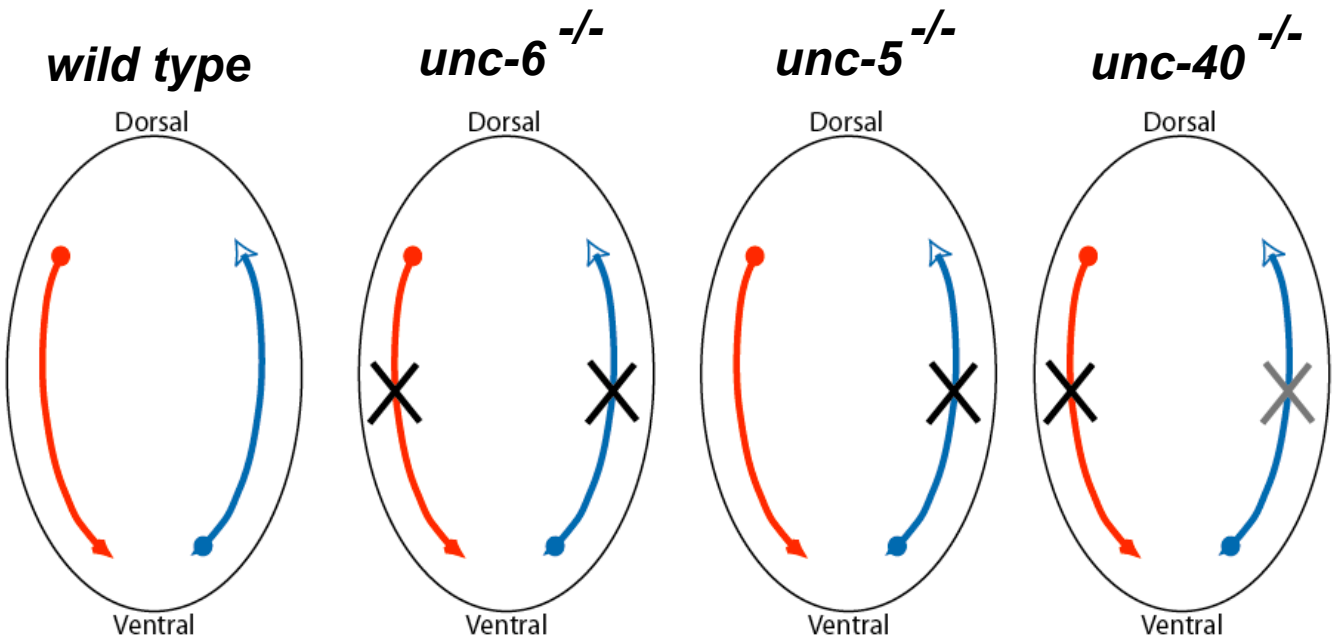
What determines whether Netrin/Unc-6 attracts or repels?

- 1) The Netrin receptors an axon expresses
  - Two Netrin receptors known:
    - » Unc-40/DCC: necessary for attraction and repulsion
    - » Unc-5: necessary for repulsion



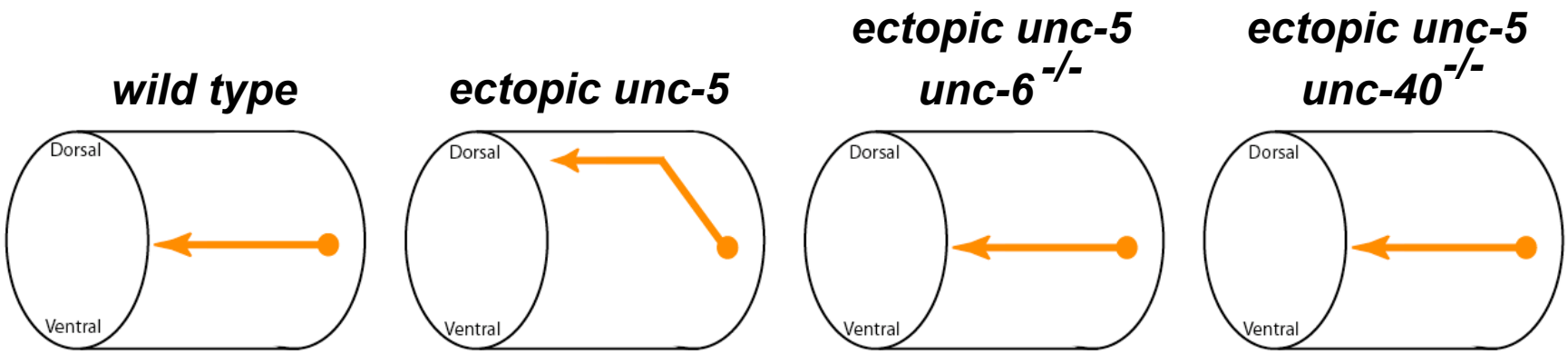
Distinct functions of netrin receptors

- **Unc-40/DCC: necessary for attraction and repulsion**
- **Unc-5: necessary for repulsion**



Ectopic expression of Unc-5 can change an axon's trajectory

- Expression of the Unc-5 receptor in neurons that normally do not express Unc-5 ("ectopic expression") can redirect axons away from the ventral midline
- Depends on Unc-6 (Netrin) and Unc-40 (co-receptor)

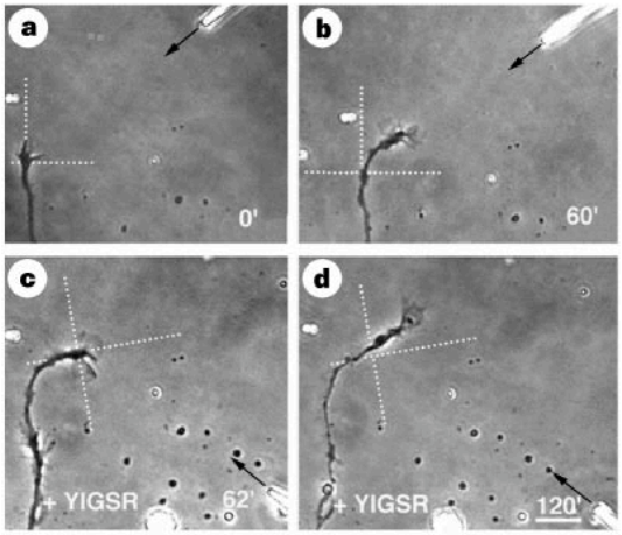


Relationship between Unc-5 and Unc-40

- Simply antagonistic? No: Unc-5 often depends on Unc-40 for its repulsive function (long-range)
- Appear to collaborate: Unc-5 and Unc-40 proteins bind to one another in Netrin-dependent fashion
- Important questions:
  - How does unc-5 convert attaction to repulsion?
  - How does unc-40 mediate attraction?
  - What are the downstream signaling pathways?

What determines whether netrin/Unc-6 attracts or repels?

- 2) The cues that are received in combination with Netrin-1



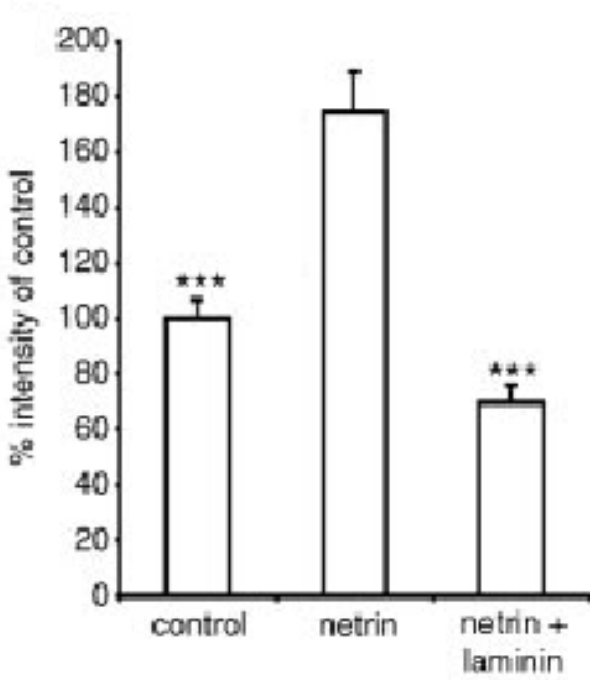
Netrin

Netrin + Laminin

- Laminin: extracellular matrix protein
  - Laminin alone neither attractant nor repellent
  - Common substrate for axons to encounter
  - Growth cones express transmembrane receptors for laminin (integrins)
- Suggests complex integration of multiple signals within growth cone

How does laminin convert attraction to repulsion?

- Laminin alters levels of cAMP in the growth cone
- Is this important?
  - Artificially elevate cAMP levels with cAMP analog, laminin no longer has effect: Netrin remains attractive
  - If inhibit cAMP signaling with drugs, Netrin repels without added laminin
- How cAMP regulates this is unknown

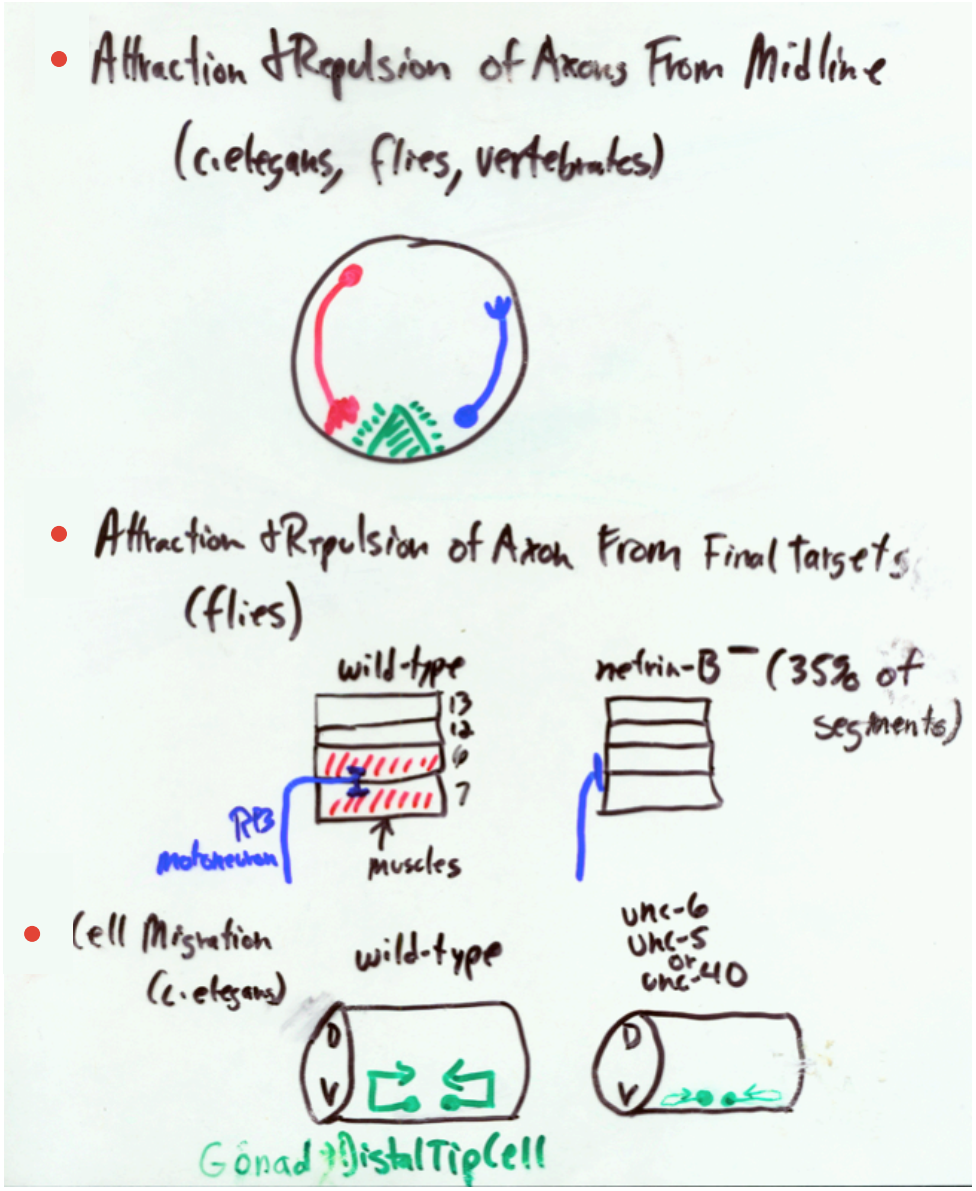


Mechanisms for determining how a growth cone responds to Netrin:

- 1) Repertoire of Netrin receptors present (unc-40, unc-5)
- 2) Identity of other guidance cues received in combination (eg., laminin)

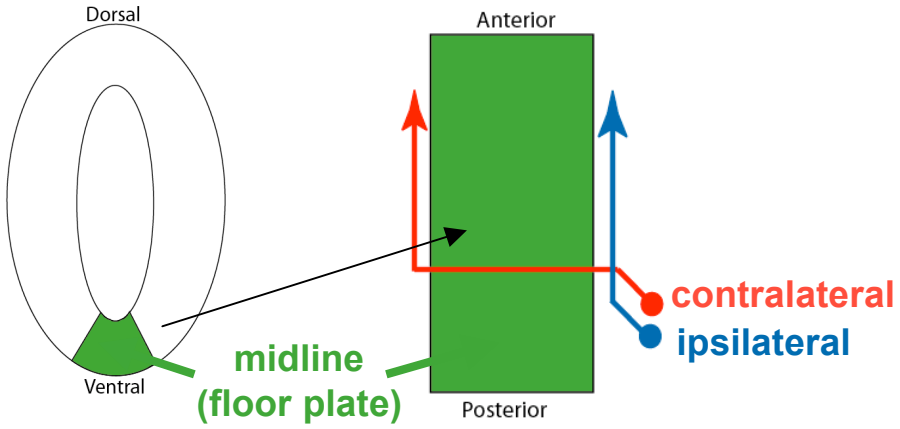
Lessons from Netrins

- A single guidance cue can mediate attraction and repulsion.
- Related proteins regulate axon guidance at the midline in worms, flies and vertebrates. Evolutionarily ancient.
- A single cue can regulate multiple steps in growth cone navigation. (In flies, Netrin is required for the RP3 motor neuron to synapse on its target muscle.)
- Axon guidance cues/receptors also cell migration. Regulate fundamental cellular constituents that affect cell motility.



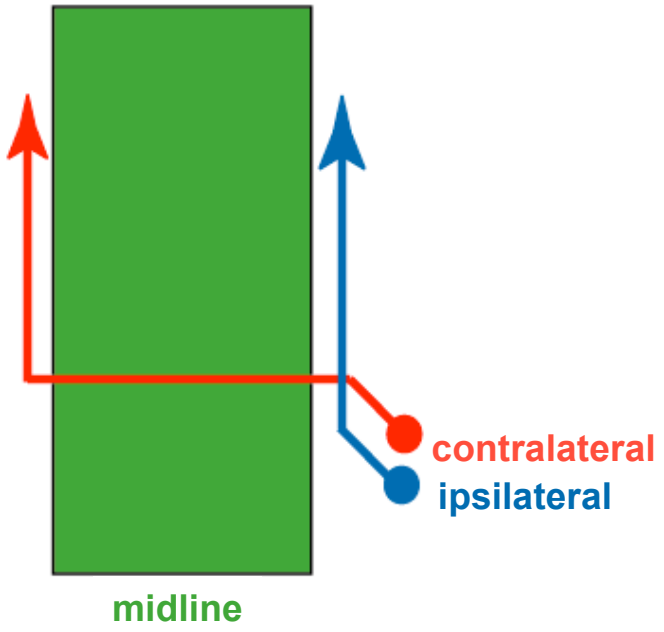
Axon guidance at the midline:

- In bilaterally symmetric animals it is important to coordinate both sides
- Netrins attract axons to the midline: what then?
- Two classes of axons:
  - Ipsilateral: don't cross midline, but grow along it
  - Contralateral: Cross midline once, then grow along it



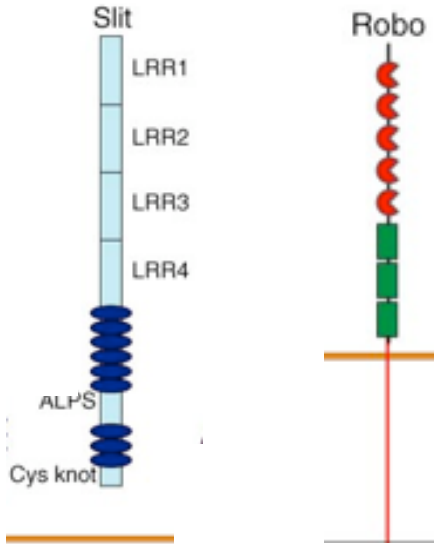
Axons at the CNS midline

- Why do axons grow along rather than within the midline?
- Why do some axons cross and others not cross?
- Why do axons only cross once?
- Partial answer: midline makes not just long-range attractant (Netrin), but also a short-range repellent.

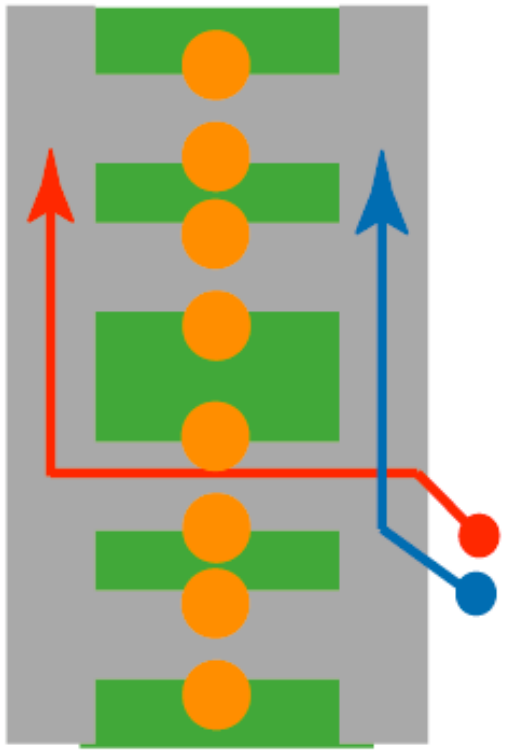


Control of axon guidance at the CNS midline

- Key insights from *Drosophila:*
  - Robo and Slit:
- Slit is a secreted protein expressed by glial cells at the CNS midline
- All CNS axons express Robo: a transmembrane receptor protein that binds to Slit
- Slit repels Robo-expressing axons *in vitro*



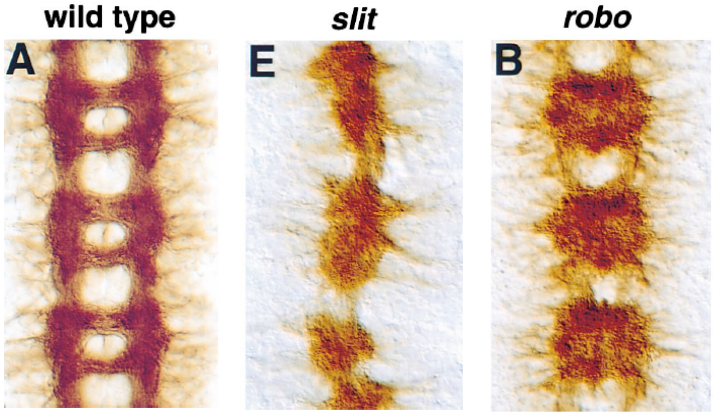
Drosophila CNS axon scaffold



midline glia
Slit

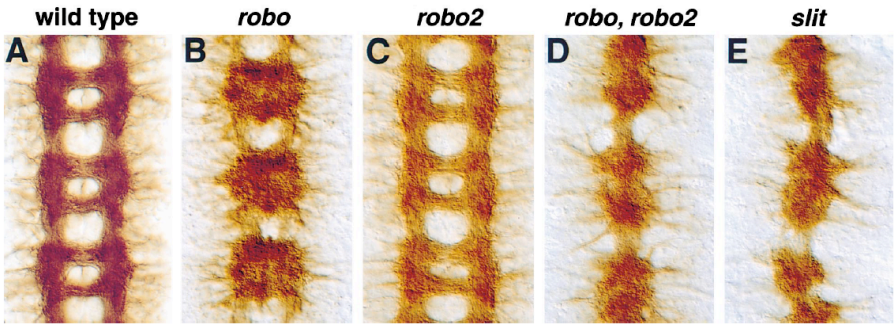
Slit and Robo at the CNS midline

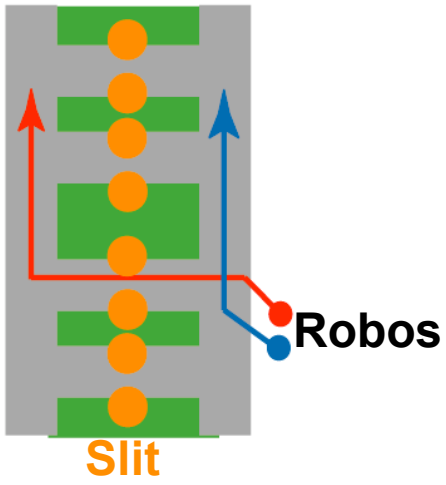
- In Slit mutants and Robo mutants axons enter the midline and don't leave
- Slit acts as midline repellent for axons expressing Robo receptor
- Why is the Robo mutant defect less severe than the Slit mutant defect?



Robo belongs to a family of receptors

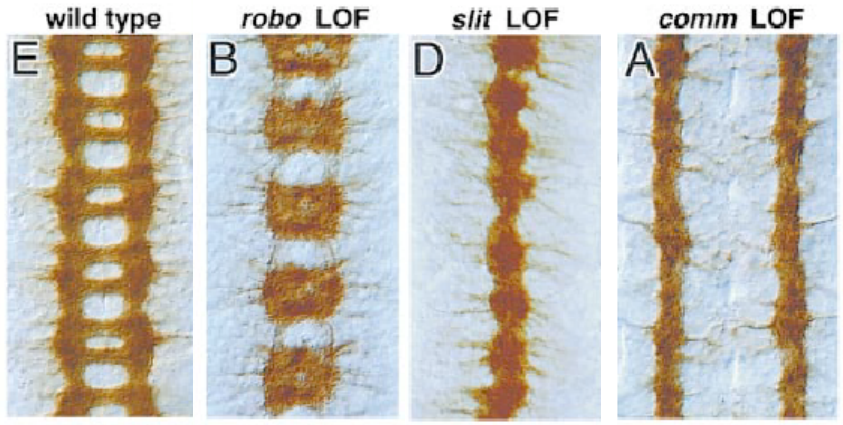
- Robo2 is closely related to Robo
- Robo and Robo2 are partially redundant
- Thus Slit/Robo signaling repels axons from the midline
- However: If Slit repels axons from the midline, how can axons ever cross?





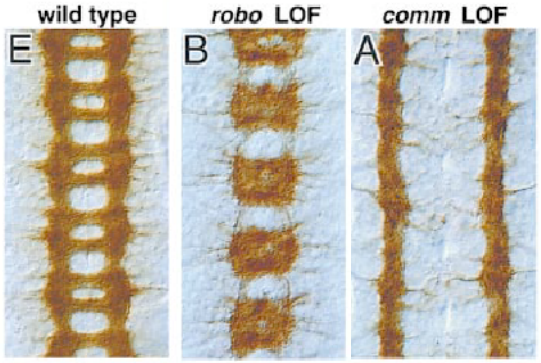
Midline signaling part 2: how do some axons cross the midline?

- **Commissureless (Comm): novel intracellular transmembrane protein**
- **Comm mutants have strong axon guidance defects: the opposite of Slit and Robo mutants**



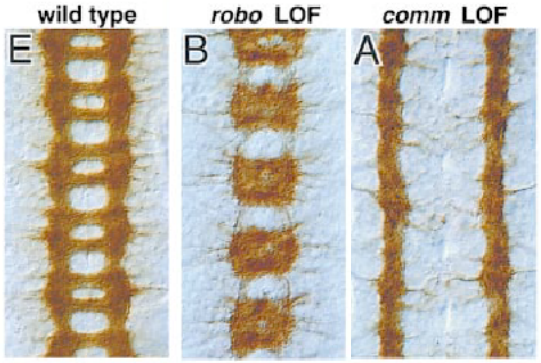
The interaction between Comm and Robo

- Test how Robo and Comm functionally interact by a genetic interaction test (epistasis).
- Way to order gene function
- Sample genetic pathway:
  - Gene A inhibits the function of Gene B
  - Mutant in gene A has opposite phenotype of mutant in gene B
  - Make an AB double mutant: what kind of phenotype do you get?



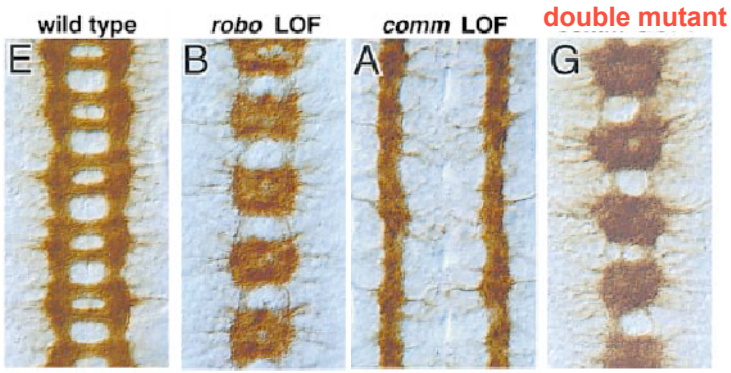
The interaction between Comm and Robo

- Test how Robo and Comm functionally interact by a genetic interaction test (epistasis).
- Way to order gene function
- Sample genetic pathway:
  - Gene A inhibits the function of Gene B
  - Mutant in gene A has opposite phenotype of mutant in gene B
  - Make an AB double mutant: what kind of phenotype do you get?
  - What would be the result if Gene B inhibited the function of Gene A instead?



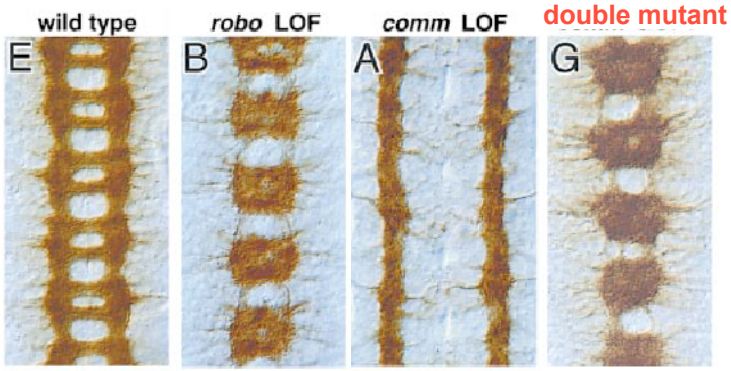
The interaction between Comm and Robo

- Robo and Comm have opposite phenotypes: consistent with Robo inhibiting Comm or Comm inhibiting Robo function
- Make comm;robo double mutant
- Double mutant resembles Robo



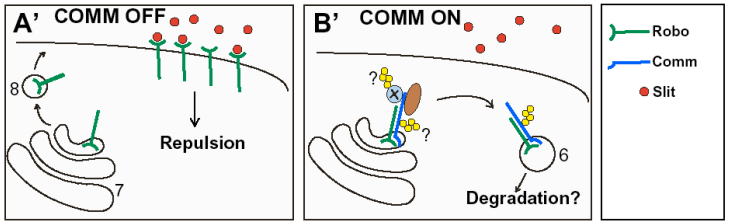
The interaction between Comm and Robo

- Robo and Comm have opposite phenotypes: consistent with Robo inhibiting Comm or Comm inhibiting Robo function
- Make comm;robo double mutant
- Double mutant resembles Robo
- Suggests: Comm negatively regulates Robo function.



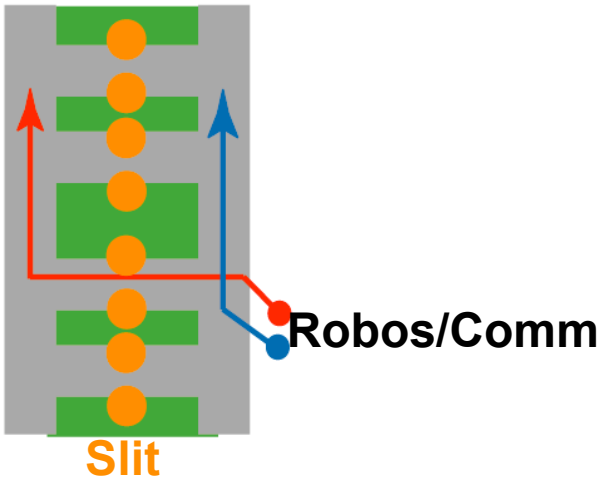
How does Comm regulate Robo?

- Comm is expressed in neurons
- Comm binds to Robo and targets it for degradation
- Thus neurons expressing Comm do not put Robo on their growth cones
- Comm-expressing neurons are not repelled from the midline



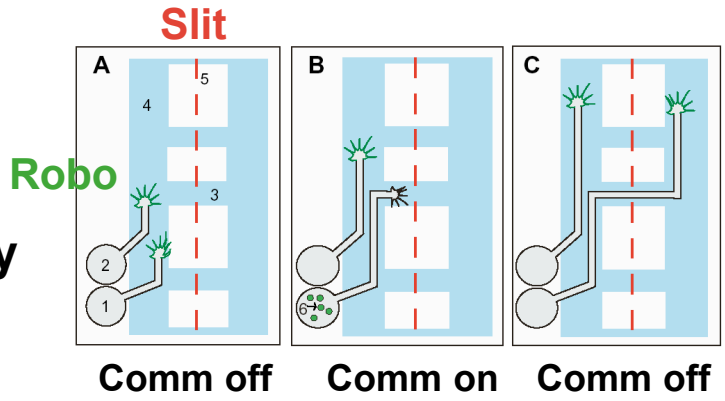
How is Comm regulated?

- The story so far:
  - Slit inhibits midline crossing by repelling Robo-expressing axons
  - Comm inhibits Robo-expression on axons permitting midline crossing
  - How are these two counteracting forces combined to generate regulated midline crossing?



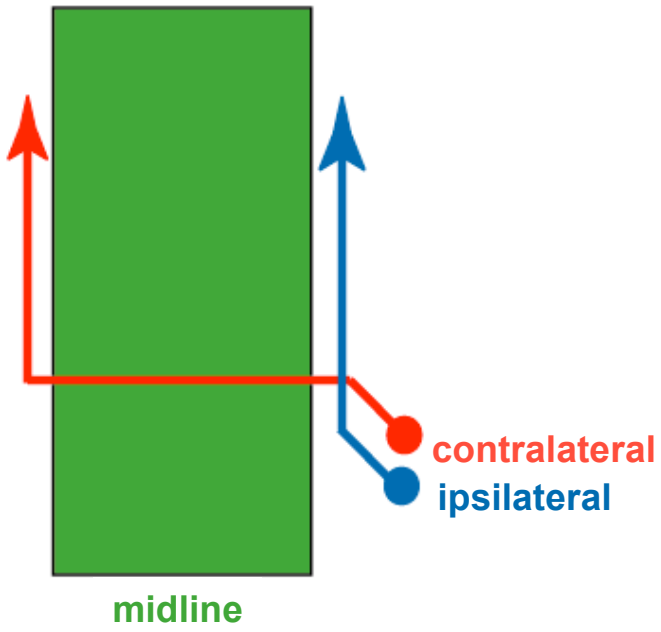
Comm regulation is key for midline guidance decisions

- How do axons enter the midline?
  - Comm expression is tightly regulated at the transcriptional level.
    - » It is only expressed in contralaterally projecting neurons transiently--- just prior to their midline crossing.
    - » It is never turned on in ipsilaterally projecting neurons.
      - Comm ON--Robo OFF -- axon enters midline (Panel B)
- How do axons ever leave the midline?
    - » Comm expression is turned off during crossing.
      - Comm OFF--Robo ON -- axon leaves midline (Panel C)

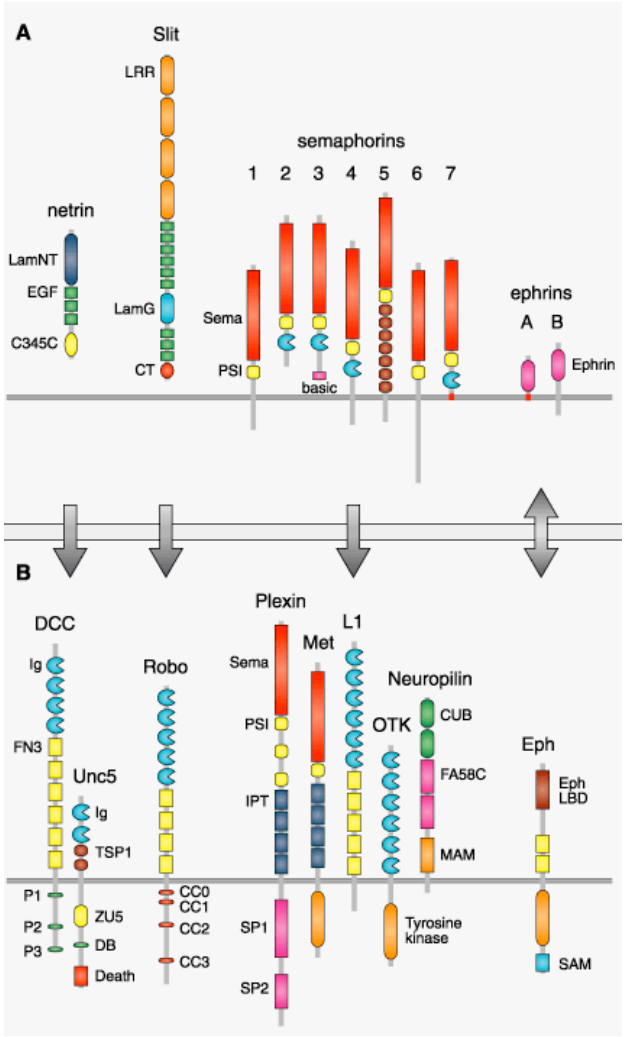


Control of midline crossing

- Combination of attractive and repulsive interactions control where axons project near the CNS midline
  - Ipsilateral axons: Never cross midline, grow along it
    - » 1) Attracted toward the midline by Netrin
    - » 2) At midline repelled by Slit
  - Contralateral axons: Cross midline once, grow along it
    - » 1) Attracted toward the midline by Netrin
    - » 2a) As approach midline, express Comm
    - » 2b) Comm inhibits Robo expression, axon ignores Slit and enters midline
    - » 3a) Neuron turns off Comm, Robo protein reaches the growth cone
    - » 3b) As Robo expression is restored, axon becomes repelled by Slit and leaves midline



Axon guidance cues and receptors



Navigation within the target region

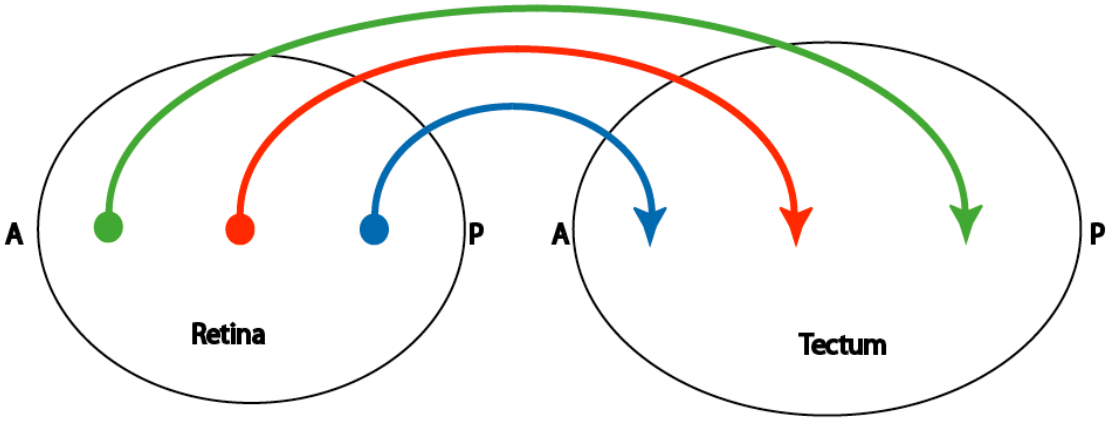
- **Axon reaches target region: still many possible target cells: How does axon choose correct one?**
  - **Topographic map formation (reach appropriate location within target field)**
  - **Post-synaptic target cell selection**

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

Retinotectal system

- Ordered by anatomical position
  - Adjacent neurons project to adjacent targets
- Thousands of retinal ganglion cells projecting to thousands of tectal targets:
  - How establish precise map?



Construction of retinotectal map

- Roger Sperry (1950's): studied frog retinotectal system
  - Cut/crush optic nerve
    - » Rotated eye 180 degrees
    - » Nerve allowed to regenerate
    - » Frog acted as if visual world upside down
  - Axons regrow to original target even if functionally inappropriate: suggested map not purely formed by neuronal activity

Construction of retinotectal map

- Sperry regeneration experiments (pt 2):
  - Cut/crush optic nerve
  - Remove half the eye
  - Axons from remaining half of eye grew to their appropriate part of tectum; rest of tectum empty (if wait a longer time, it's more complicated)
  - Suggests: Recognition between axon and target
- 1963: Sperry proposed Chemoaffinity hypothesis
  - Axon and target cells have selective chemical affinities for one another

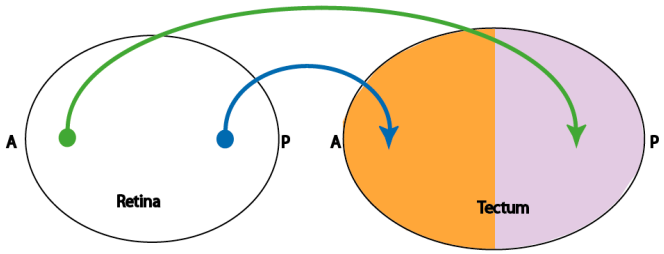
Sperry chemoaffinity model

- Thousands of axons and targets: will each axon/target pair have its own unique, complementary label?
- No --- Sperry predicted existence of complementary gradients of signaling molecules on axons and targets:
  - Rationale:
  - Economical: only a few molecules needed
  - Act over large region: Gradients could be sensed throughout target. If axon is in wrong place could tell which way to go toward target. It would not be just a random search for a match.
- Are there actually such gradients? If so, gradients of what?

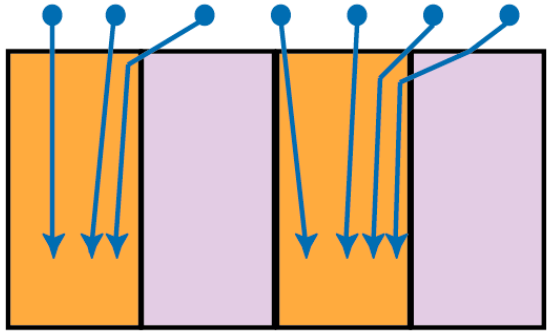
Assaying for gradients of chemoaffinity molecules

- **70's and 80's: *In vitro* assays demonstrated activities consistent with chemoaffinity**
- **Bonhoeffer devised tectal membrane "stripe assay"**

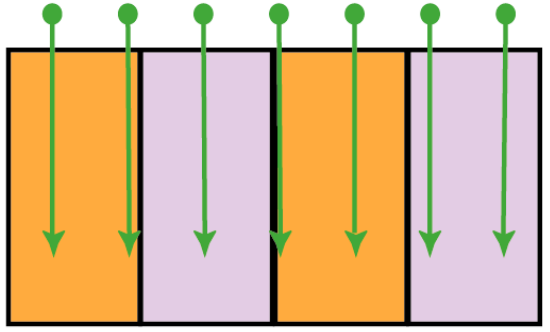
Stripe Assay: Prepare membranes from anterior and posterior tectum



Posterior axons avoid posterior tectal membranes

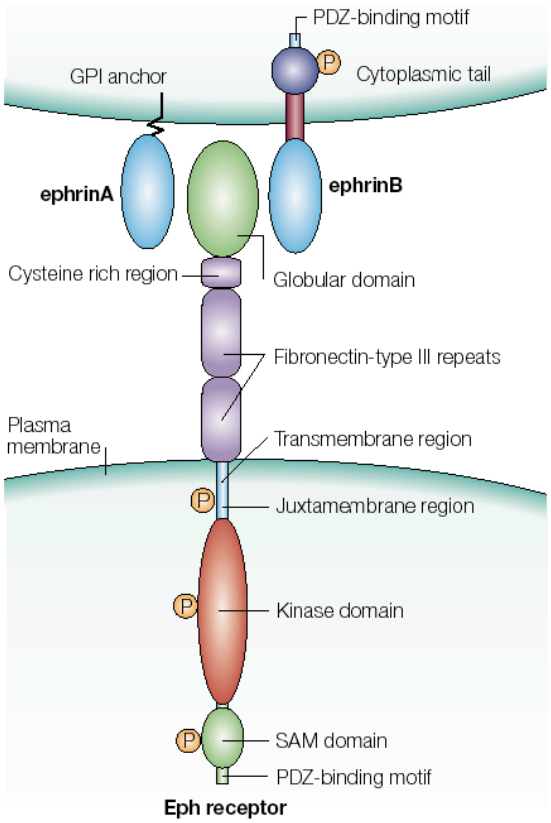


Anterior axons don't care



1995: first “chemoaffinity” molecules identified

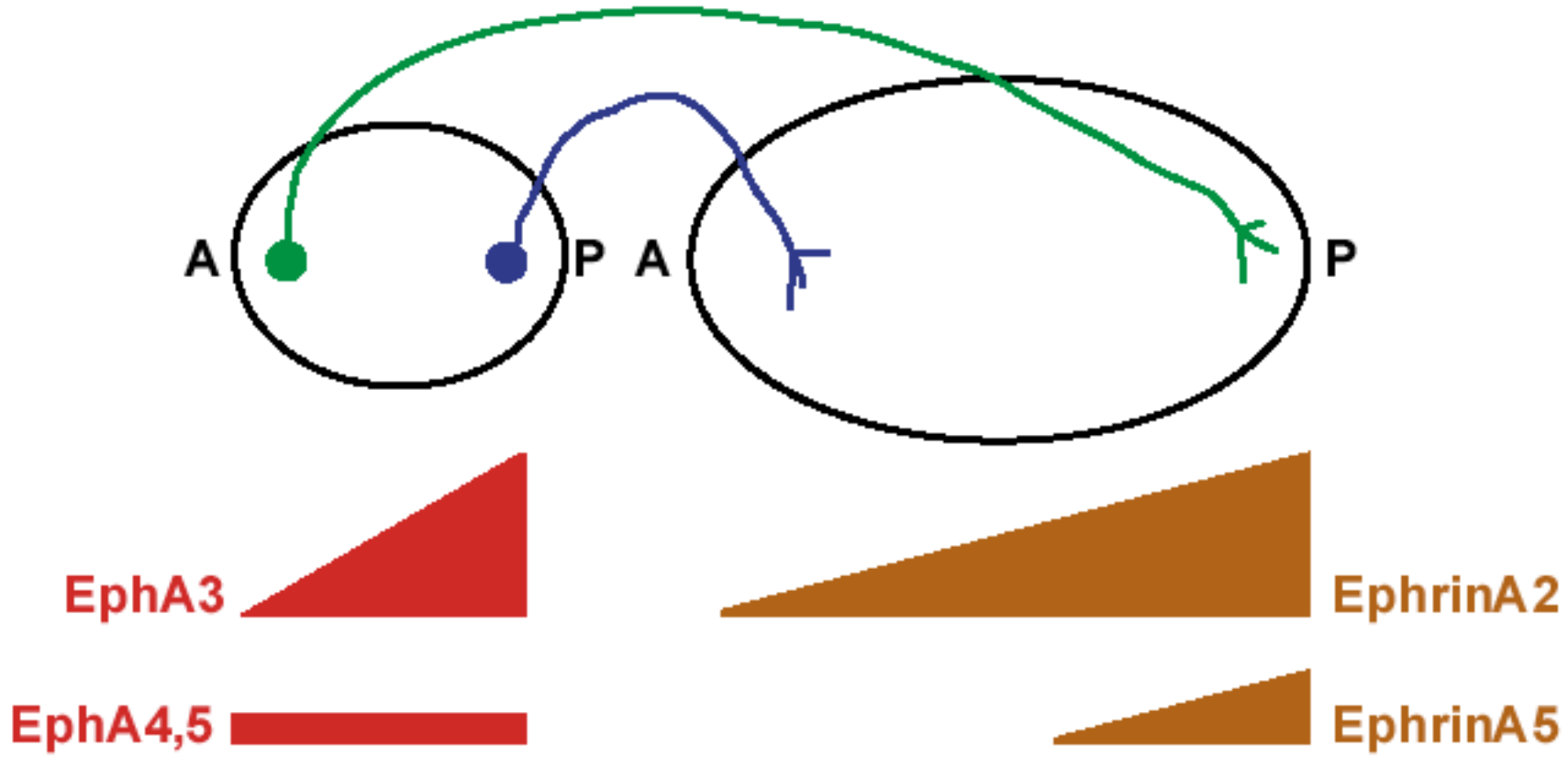
- Eph receptors: transmembrane receptor tyrosine kinases
- Ephrins: bind Eph receptors
  - Class A: GPI-anchored
  - Class B: transmembrane



Eph/Ephrin

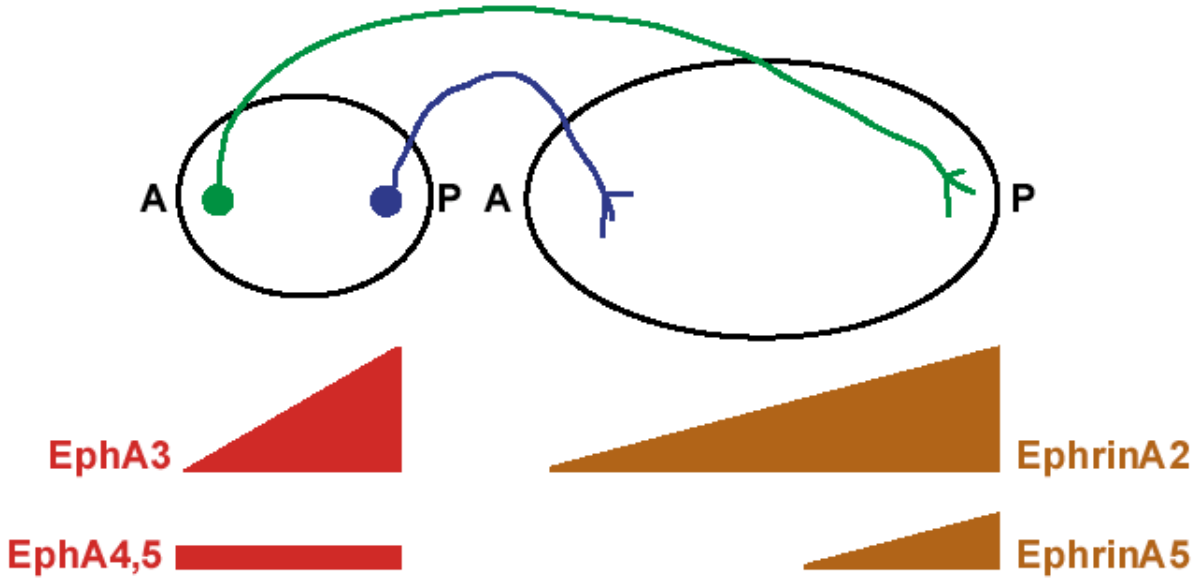
- **Basic rules:**
  - **EphA bind EphrinA**
  - **EphB bind EphrinB**
- **Eph's are unusual receptor tyrosine kinases: require clustering of ligand**
  - **Likely to require cell-cell contact, hence highly localized signaling**
- **Known to mediate axon repulsion**

Eph and Ephrins in the Retinotectal System

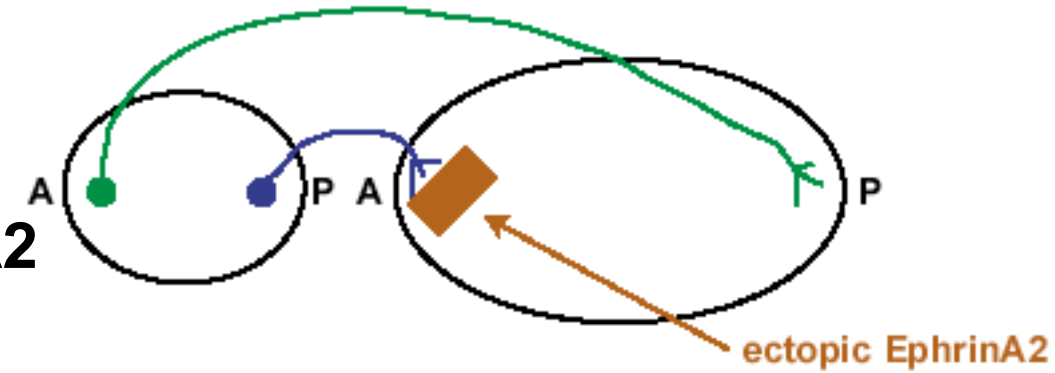


- Expressed in complementary gradients on axons/targets.

Ephrins can mediate topographically specific repulsion



- *In vitro*: ephrins repels P but not A axons.
- *In vivo*: ectopic Ephrin A2 causes posterior but not anterior axons to stop short.



Ephs and Ephrins in map formation

- Gradients of Ephs and Ephrins found in many brain regions where topographic maps form
- How do they determine placement of axon within a map?
  - One model: [Eph]x[Ephrin] = repulsive force : axons move until hit threshold of repulsion
  - However: Knockouts seem to randomize map
    - » don't cause all axons to go to one extreme

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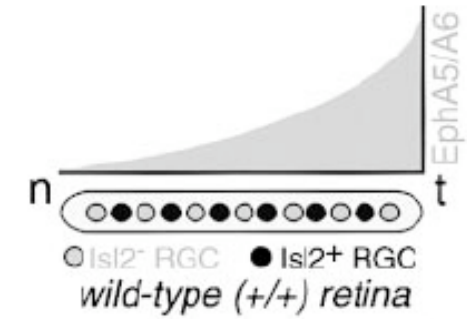
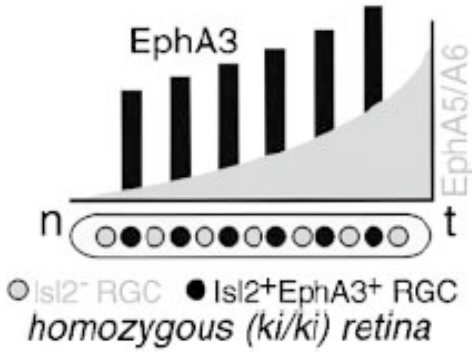
    - » don't cause all axons to go to one extreme
  - Second model: Relative level of signaling compared to other axons is key

Evidence for relative level model

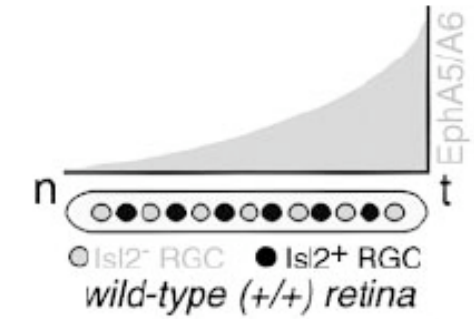
- Put EphA3 receptor into locus expressed in ≈50% of retinal ganglion cells (Isl2)
- If absolute signaling is key: Isl2$^-$ axons target normally
- If relative level of signaling is key: Isl2$^-$ axons shift and two separate maps form: one for Isl2$^-$ and one for Isl2$^+$ axons

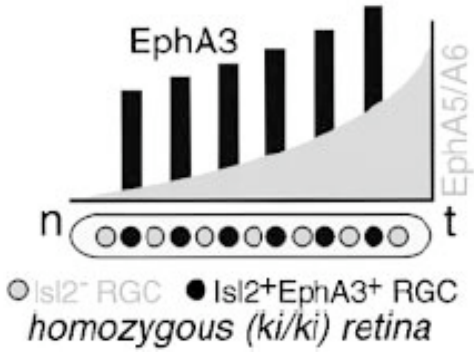




Result: two maps form

- Isl2^{-} axons form map over posterior half of target
- Isl2^{+} axons form map over anterior half of target
- Suggests relative level of Eph signaling is key





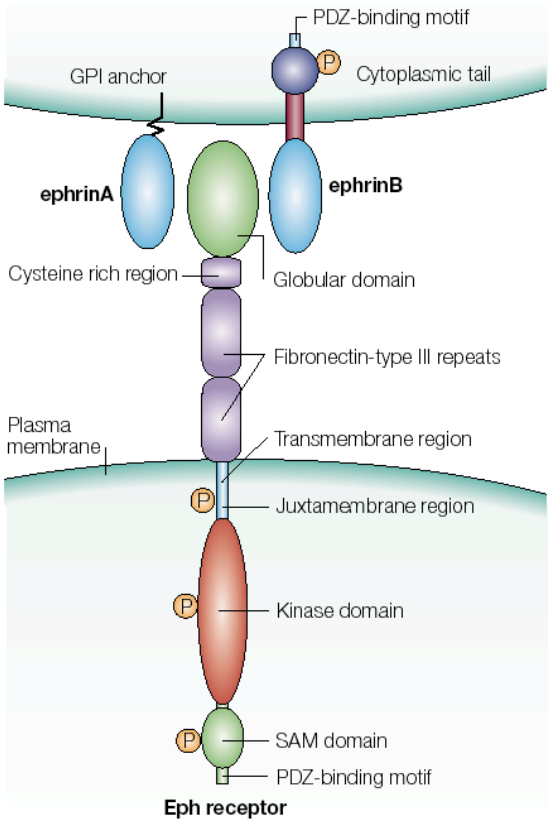
Ephrins and Ephs: Who is the ligand and who is the receptor?

- EphB2 null mouse: Axons of posterior tract on anterior commissure make pathfinding errors.

- However... EphB2 mutant where extracellular domain present, but no kinase => No Defect!!

- Look at expression:
  Ephrin Bs are expressed on axons
  EphB2 expressed on SUBSTRATE!

Is EphB2 a ligand for Ephrin Bs?



Ephrins and Ephs: Who is the ligand and who is the receptor?

- Can EphB2 act as a ligand for Ephrin Bs?

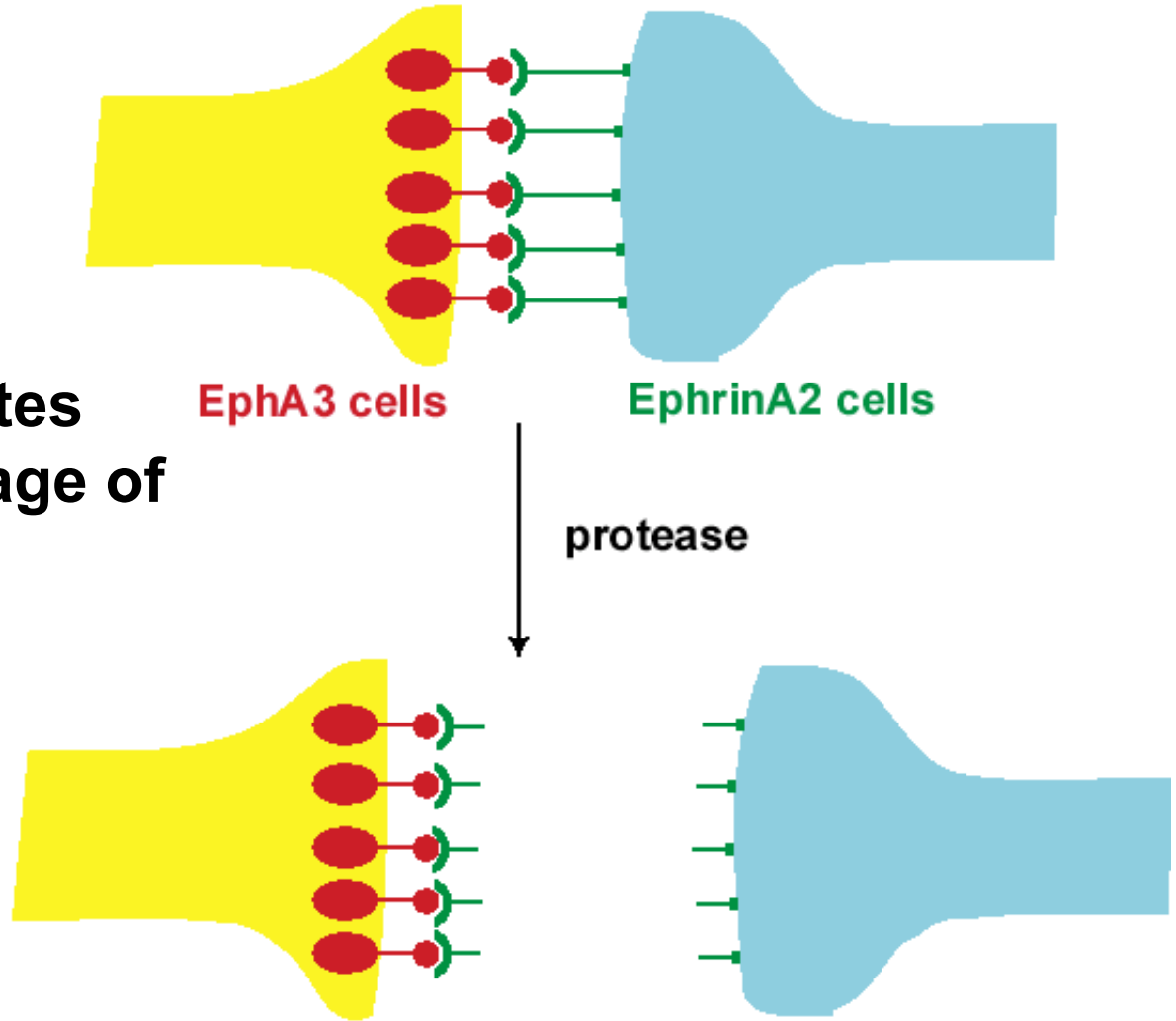
- Binding of EphB2 to EphrinB1 causes the intracellular domain of Ephrin B1 to become tyrosine phorphrylated.

=> EphBs and Ephrin Bs can mediate bidirectional signals

- Provides a way for coordinating the response of two interacting cell populations.

The Eph/Ephrin Interaction:
How can the interaction between membrane-associated proteins result in repulsion?

- **EphA3 binding to Ephrin A2 promotes proteolytic cleavage of Ephrin A2.**

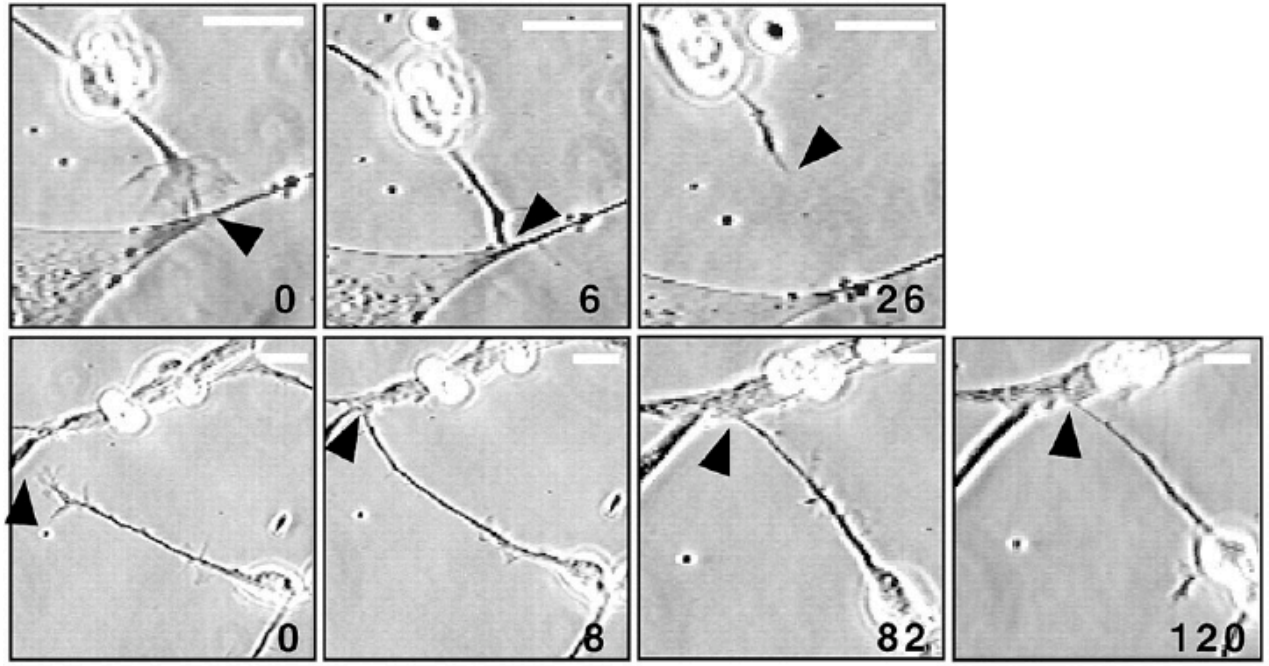


The Eph/Ephrin Interaction:

How can the interaction between two membrane-associated proteins result in repulsion?

Cultured cell: EphrinA2 (wt)

Cultured cell: EphrinA2 (mut)



- **Uncleavable form of EphrinA2 : Growth cone collapses but doesn't withdraw.**
- **Proteolysis permits withdrawal (and redirection).**

Next time:
Target selection (continued)
Cell biology of growth cone navigation