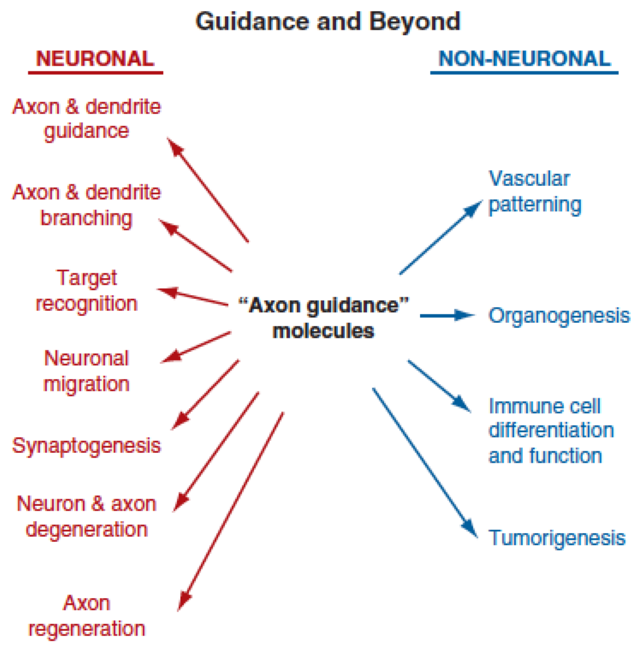
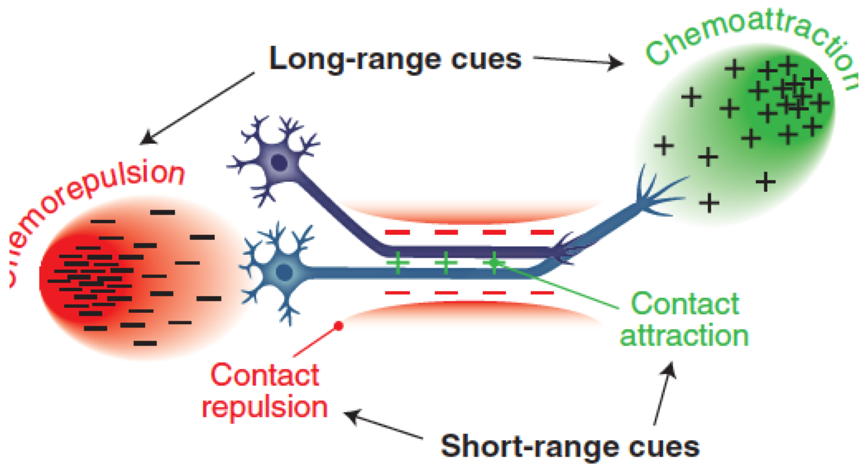
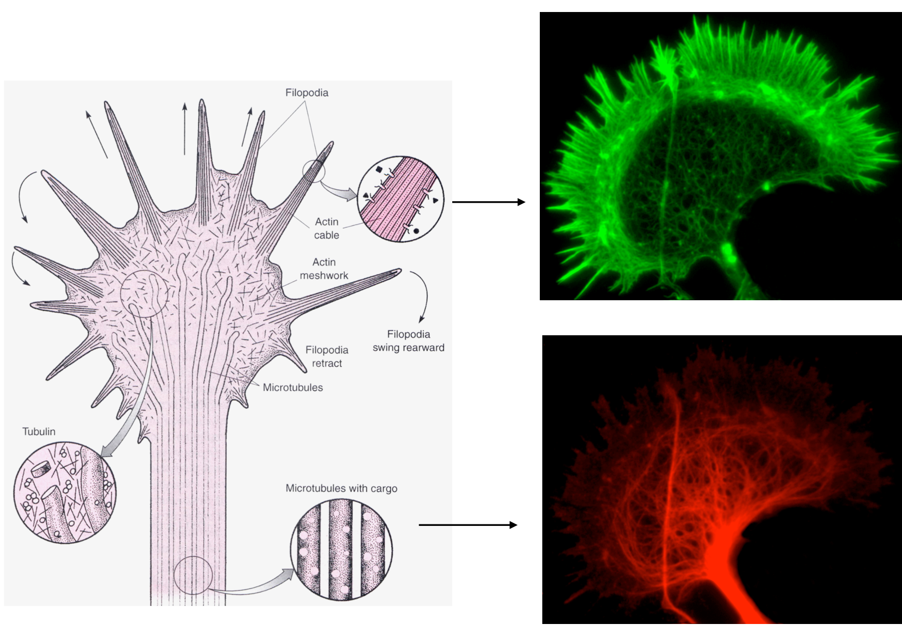
Axon guidance



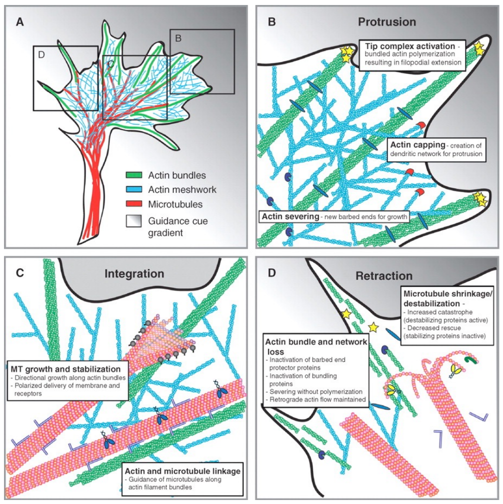
Guidance cues

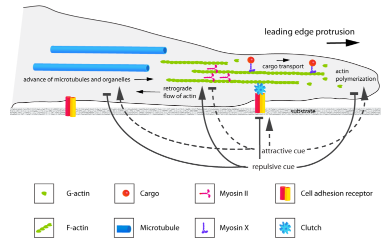


The growth cone



Coordinating actin and microtubule dynamics in growth cone turning





Dent E W et al. Cold Spring Harb Perspect Biol 2011;3:a001800

Guidance Cue

Receptor

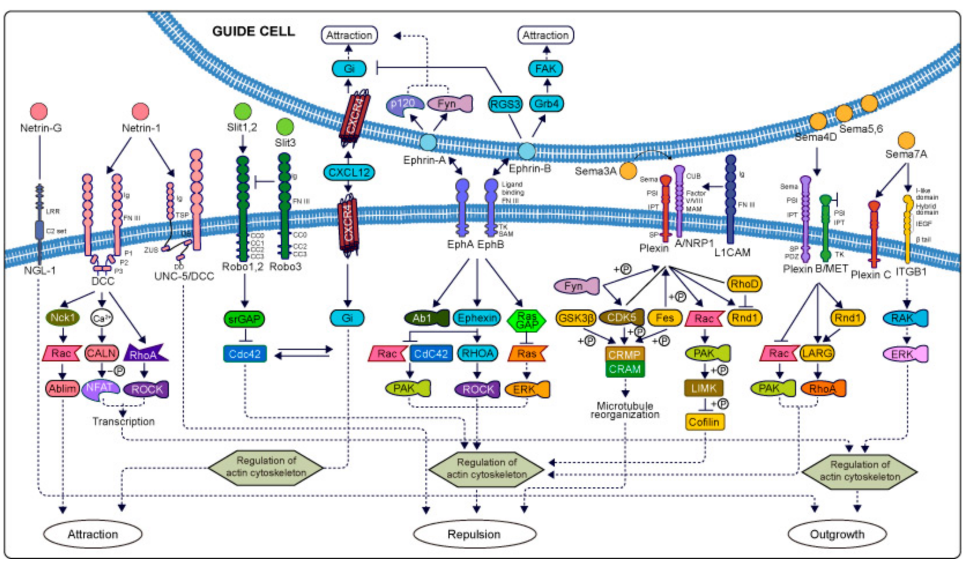
Signal

Target

Cytoskeleton

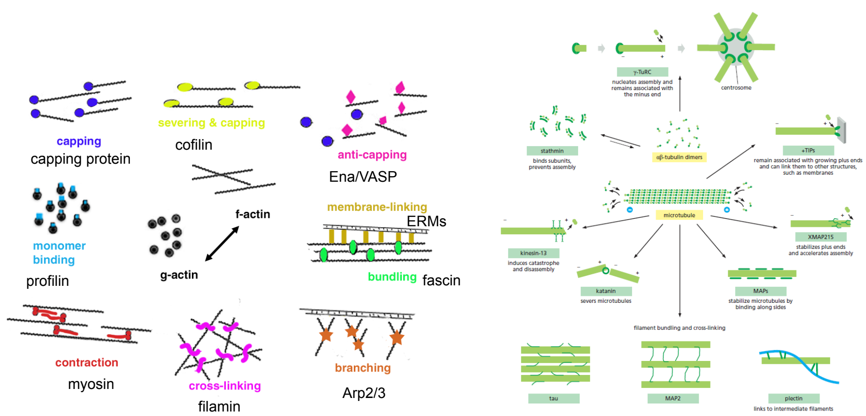
Growth Cone Behavior

Axon guidance pathways connect to the cytoskeleton



http://www.genecopoeia.com/product/search/pathway/h_axonPathway.php

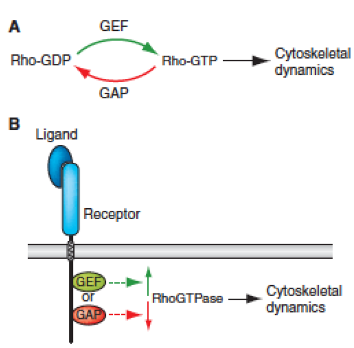
Axon guidance signals converge on actin- and microtubule-binding proteins

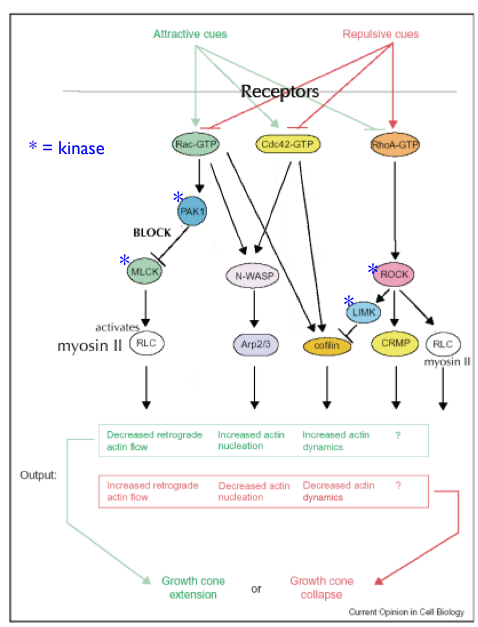


- Axon guidance signals converge on actin- and microtubule-binding proteins

- Functional outcome depends on
  - localization
  - relative concentration of other regulatory proteins

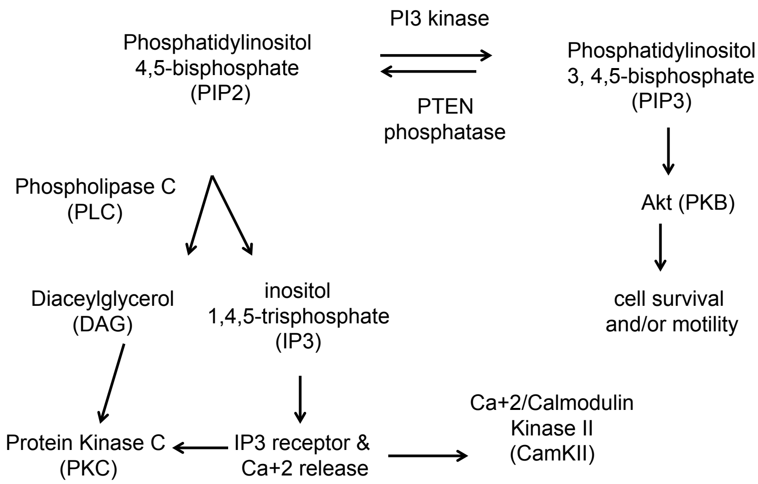
Rho, Rac & cdc42: collapse vs. extension



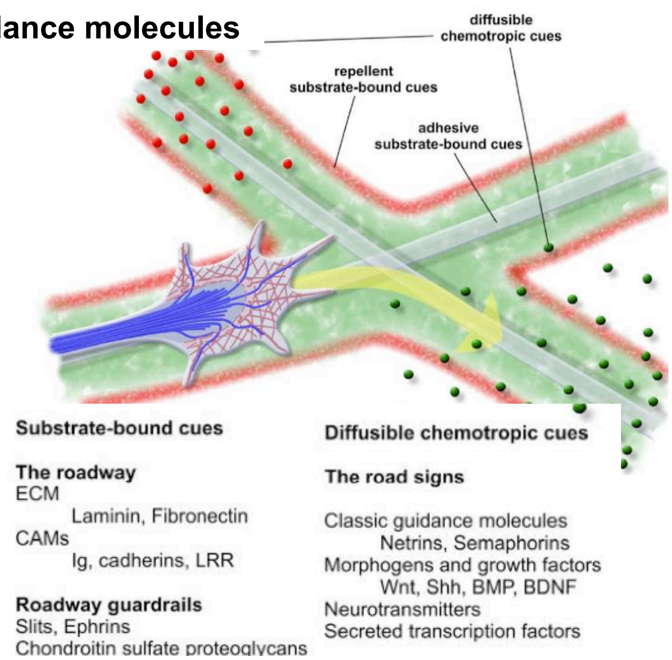


- an activation/inhibition step may be 1:1 or can amplify the signal

Lipid phosphatase, kinases and lipase



Guidance molecules

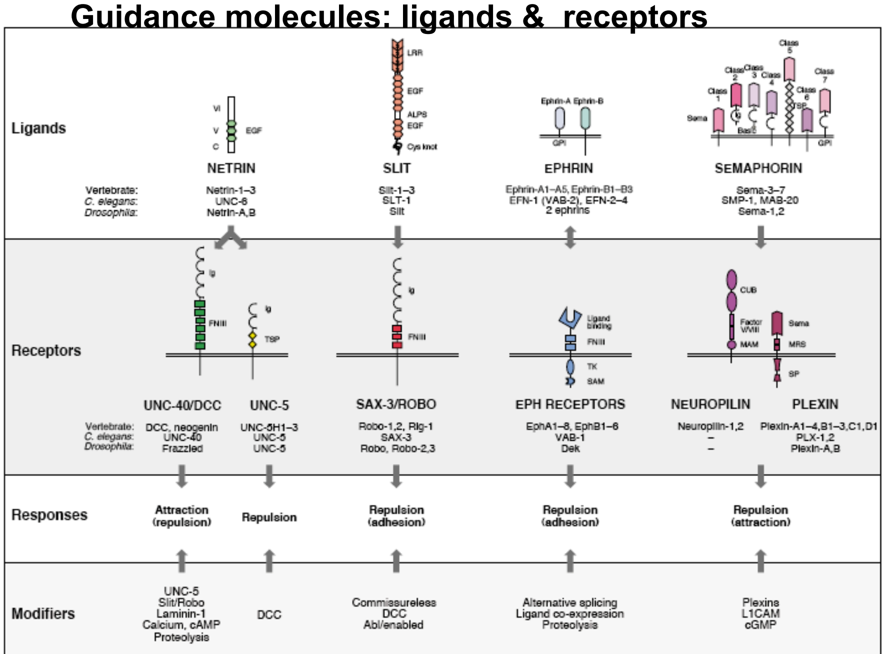


Lowery and Van vactor (2009) Nat Rev Mol Cell Biol. 10(5): 332–343

Axon guidance signals

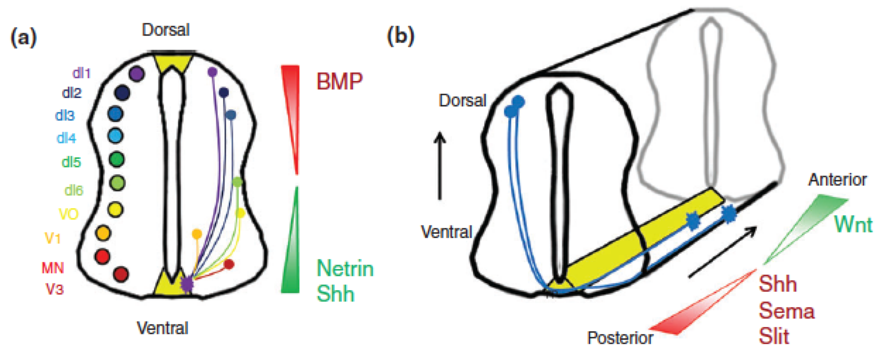
- Requires spatial and temporal specificity of expression of guidance receptors and ligands
- Guidance molecules usually work in combinations
- Show significant cross-talk, with effects that can be
  - Parallel
    - EphrinAs and EphAs/GDNF guidance of L-MCL towards the dorsal limb
  - Hierarchical
    - Silencing of netrin-1/DCC attraction by Slit/Robo
  - Synergistic
    - Guidance of LMCL axons to the dorsal limb by GDNF and EphAs acting through the common signaling receptor Ret
  - Permissive
    - Post-crossing commissural axons encounter midline NrCAM, GDNF, and Shh, which switch on axon responsiveness to Sema3s
- Functions can be modified by the local environment

Guidance molecules: ligands & receptors



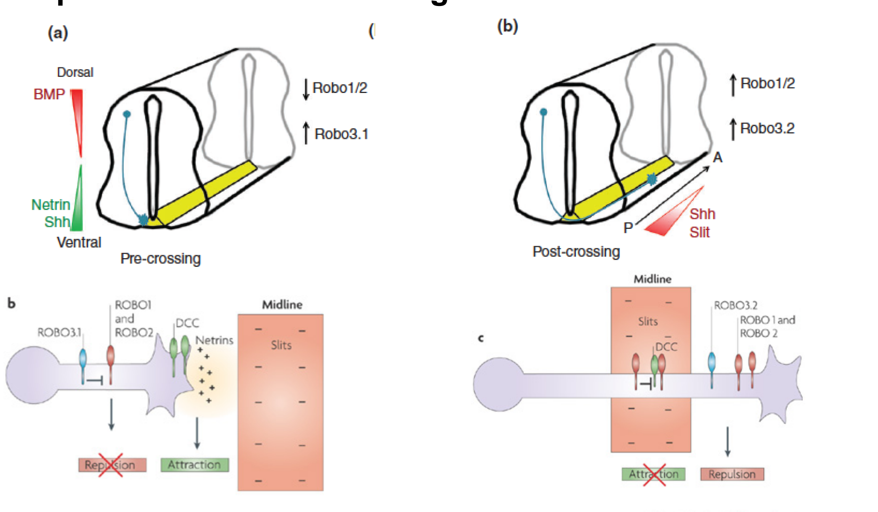
Yu and Bargman (2001) Nature Neurosci.

Vertebrate spinal commissural axon guidance



Martinez and Tran. WIREs Dev Biol 2015. doi: 10.1002/wdev.173

Spinal commissural axon guidance: Anterior/Posterior



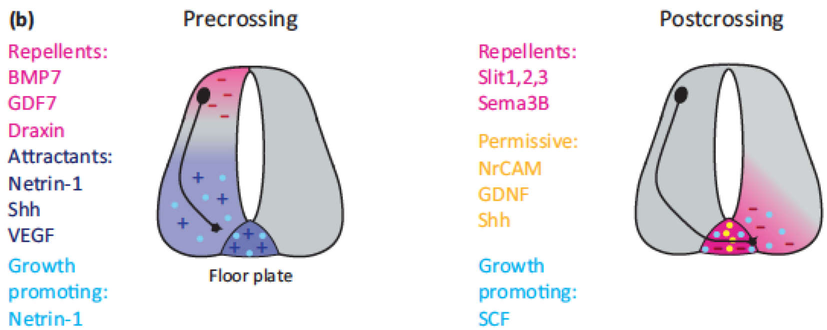
- DCC receptor mediates attraction to floorpate Netrin
- Robo 3.1 silences Robo 1 & 2

- DCC receptor silenced by Robo 1 & 2
- Robo 1, 2 & 3.2 mediate repulsion to midline slits

Daniel L. Chao, Le Ma & Kang Shen
Nature Reviews Neuroscience **10**, 262-271

Martinez and Tran. WIREs Dev Biol 2015. doi: 10.1002/wdev.173

Spinal commissural axon guidance: midline crossing

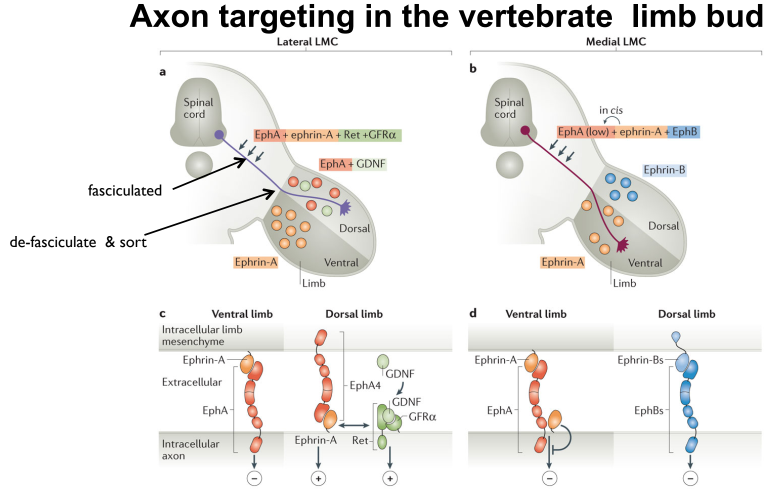


- Commissural axons are first guided towards the floor plate by dorsally expressed repellents (pink gradient)
- Ventrally expressed attractants (blue gradient) and growth-promoting factors (light blue dots) guide axons to the floor plate

- Exposure to permissive factors (yellow dots), sensitizes axons to midline repellents
- Repulsive cues (pink) then expel axons from the floor plate
- Stem cell factor (SCF; light blue dots) stimulates axon extension

Dudanova, I., and Klein, R. (2013). Trends Neurosci 36, 295–304

Axon targeting in the vertebrate limb bud

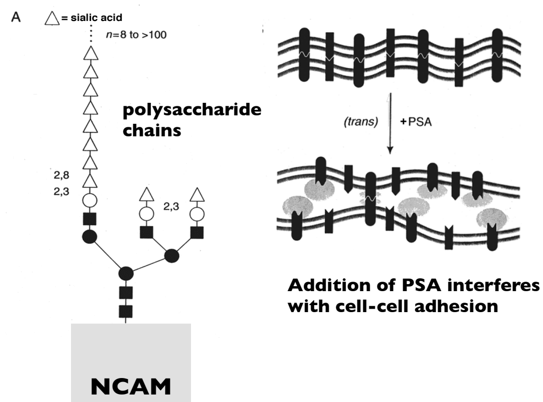


- L-LMC axons express EphA and are repulsed by Ephrin A in ventral limb
- L-LMC axons express EphrinA and Ret/GFRa and are attracted to EphA and GDNF in the dorsal limb

- M-LMC axons express EphB and are repulsed by Ephrin B on the dorsal limb
- M-LMC axons express both EphA and EphrinA. When they encounter EpherinA in the ventral limb, EphA-mediated repulsion is silenced

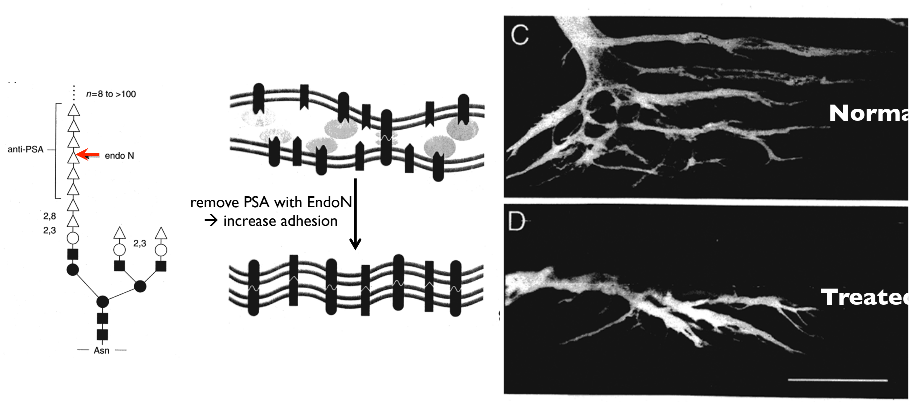
What causes de-fasciculation at the limb bud?

- NCAM on axons promotes fasciculation
- Linking of highly negatively charged sialic acid sugars (PSA) on the extracellular polysaccharides of NCAM decreases axon-axon adhesion to promote de-fasciculation of axons.



Rutishauser, U., and Landmesser, L. (1996). Trends Neurosci 19, 422–427.

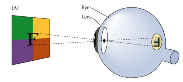
Enzymatic removal of PSA disrupts axon de-fasciculation

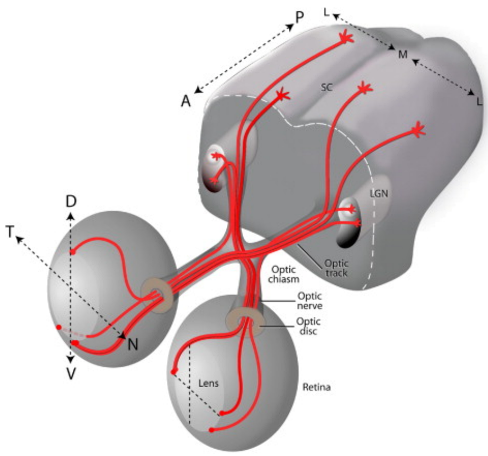


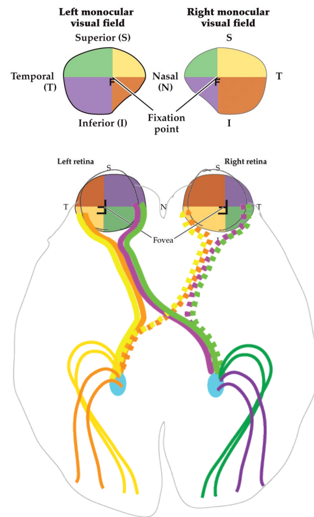
Failure to defasciculate

Rutishauser, U., and Landmesser, L. (1996). Trends Neurosci 19, 422–427.

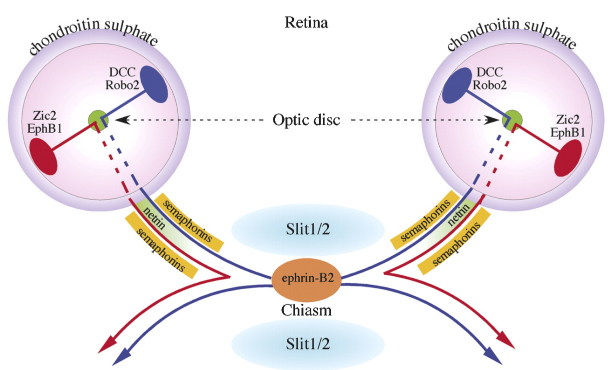
Retinal axon protections







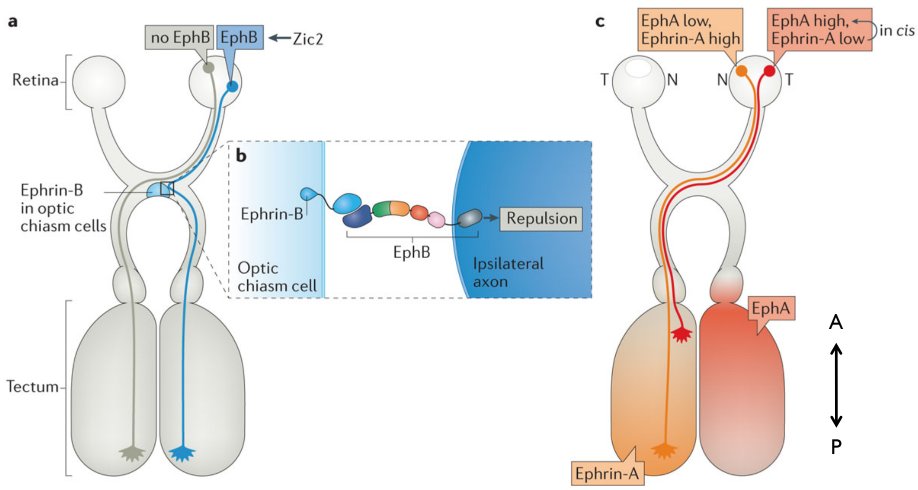
RGC axon guidance across the chiasm



- In the retina, axons are repelled from the periphery by chondroitin sulfate.
- At the optic disc, RGC axons exit the retina into the optic nerve using a mechanism based on attractive netrin/DCC-mediated action.
- Within the optic nerve, RGC axons are kept within the pathway through semaphorin distribution and by inhibitory Slit/Robo interaction. Slits also contribute to positioning the optic chiasm by creating zones of inhibition.
- Zic2-expressing RGCs in the VT retina project EphB1-expressing axons, which are repelled by ephrin-B2 at the optic chiasm and terminate in ipsilateral targets.

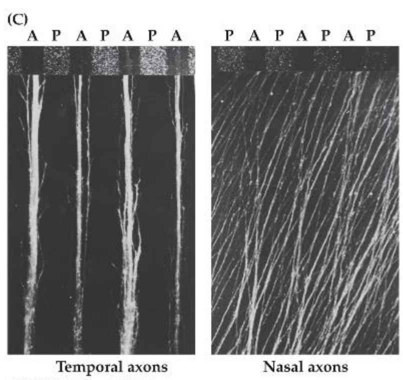
Takayuki Harada et al. Genes Dev. 2007;21:367-378

RGC axon guidance along the A-P axis of the tectum

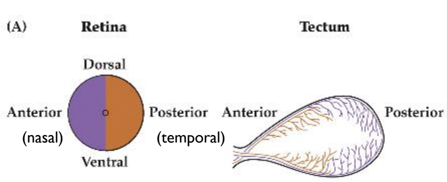


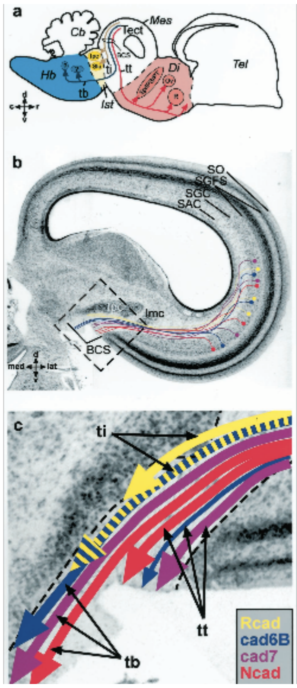
Kania & Klein *Nature Reviews Molecular Cell Biology* (2016)

An *in vitro* assay for axon guidance



- In the stripe assay, membrane proteins from parts of the tectum are striped onto a dish, then ganglion cells from the retina are placed at one end of the stripes and their axons allowed to grow.
- Temporal axons
  - grow on membrane from the anterior tectum
  - avoid membrane from the posterior tectum
- Nasal axon show no preference





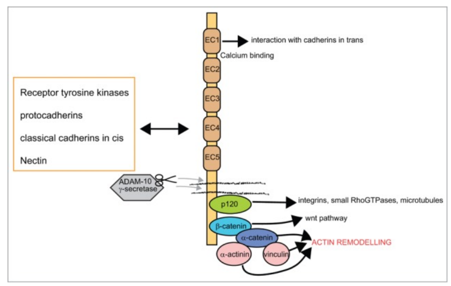
Treubert-Zimmermann et al. (2002) J. Neurosci.

Sorting of axonal projections from the optic tectum is regulated by adhesion molecules

In vertebrates (Xenopus Laevis):

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; cadherin7, cadherin6B,N-cadherin, R-cadherin

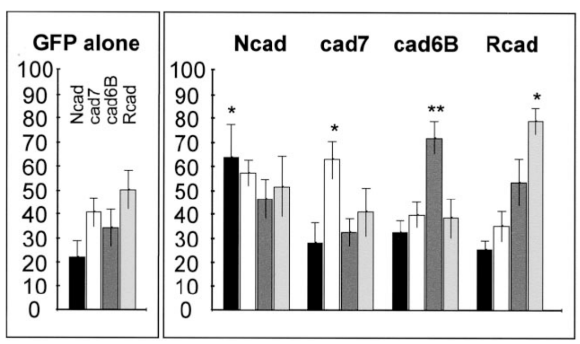


Cell Adh Migr. 2015 May-Jun; 9(3): 175–182.

Adhesion molecules regulate sorting of axons to specific targets

Transgene expressed by tectal axons

Level of specific cadherin expression by neighboring axons

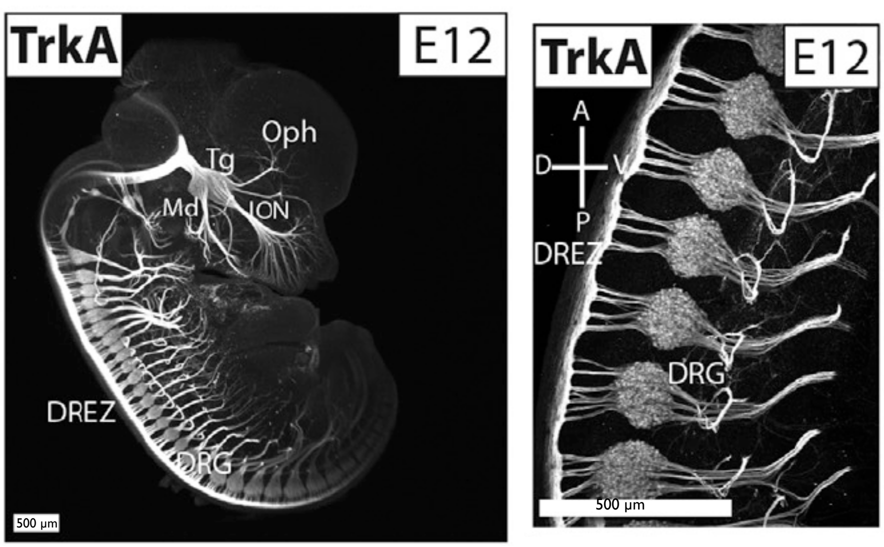


Electroporation of optic tectum to force expression of each of these four cadherins causes the transfected axons to preferentially travel near axons carrying the same cadherin

Treubert-Zimmermann et al. (2002) J. Neurosci.

DRG sensory axon targeting

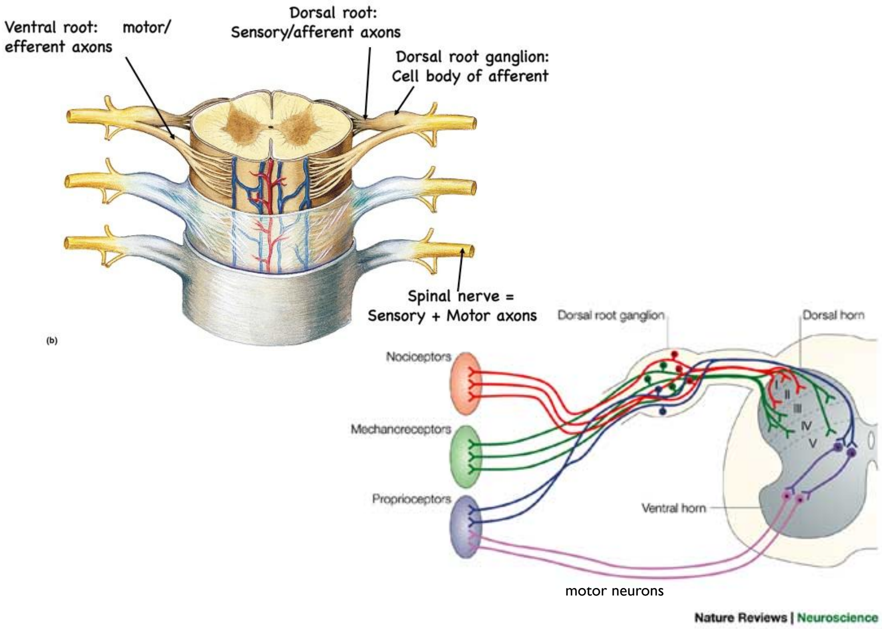
Dorsal root ganglia



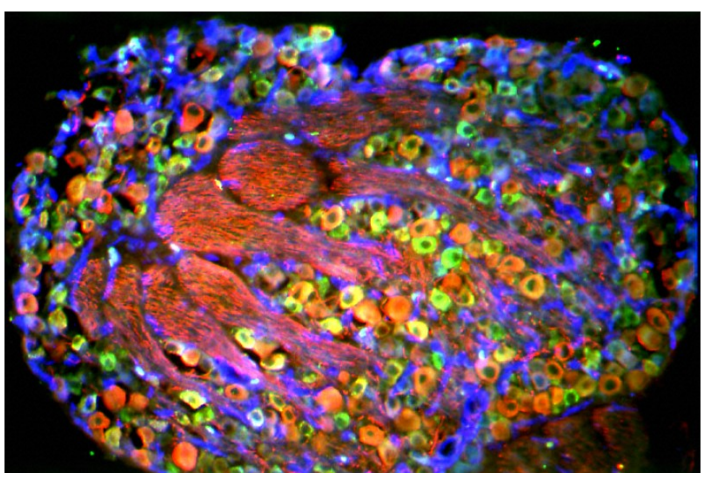
Nociceptive TrkA expressing neurons

Cell 159, 1–15, November 6, 2014

What are the dorsal root ganglia (DRGs)?

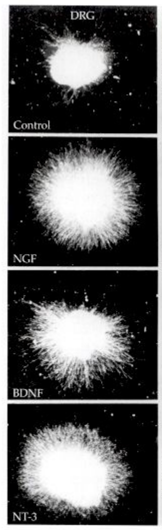


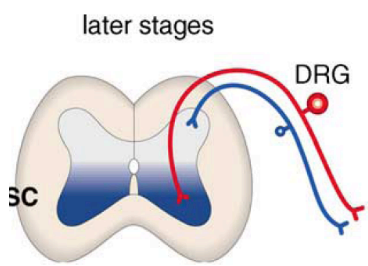
The DRG contains many types of cells



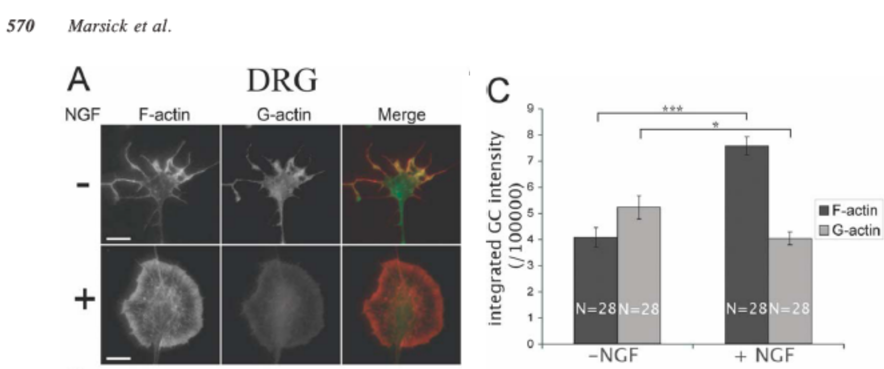
Wellcome Images **Pain and touch sensory fibres in DRG Section** through a neonatal dorsal root ganglion (DRG) showing the cell bodies of different populations of sensory nerves. The red stains the myelinated A fibres that detect tactile sensations (proprioception). The green stain is specific to peptidergic neurons - these detect pain and mediate neurogenic inflammation via the release of certain peptides both at the painful site and in the spinal cord (nociception). The blue stain is specific to the non-peptidergic pain neurons (nociception) . Bundles of nerve fibres can also be seen within the ganglion. **B0003822** Credit Simon Beggs,

Several neurotrophins can enhance (total) DRG axon outgrowth,
but it is actually different populations of neurons affected





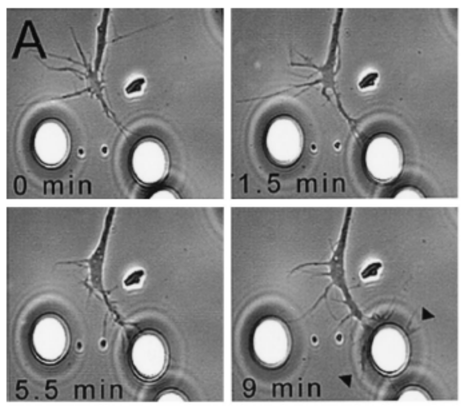
Acute exposure of DRGs to NGF
induces changes in the actin cytoskeleton



DRGs grown in very low NGF, then exposed to high NGF

Acute test:
A directional source of NGF induces DRG growth cone turning

Gallo et al. • trkA Mediates Growth Cone Turns toward NGF Beads

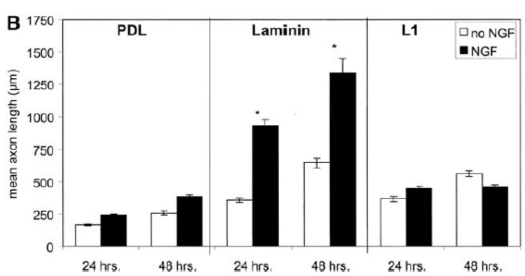


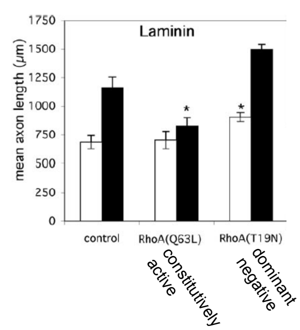
5450 J. Neurosci., July 15, 1997, 17(14):5445–5454

Table 1. Relative decrease in growth cone turning frequency toward NGF-coated beads

Treatment or NGF background concentration	(n)	Relative percentage of growth cones turning (RPT)
0.05 ng/ml NGF	27	100%
1.0 ng/ml NGF	18	50
10.0 ng/ml NGF	20	17
100 ng/ml NGF	15	0
25 μg/ml anti-trkA	17	0
14 μg/ml anti-p75 antibodies	17	65
70 μg/ml anti-p75 antibodies	16	62
20 μg/ml anti-trkB	13	85
25 μg/ml anti-L1	12	96
10.0 ng/ml BDNF	10	88
100 ng/ml BDNF	16	50
100 nM K252a	19	0 (CaMK inhibitor)
100 nM KT5926	8	92 (MLCK inhibitor)
500 nM KT5926	15	55
2.5 μl/ml DMSO	8	92

Chronic test:
The trophic effects of NGF on axon outgrowth
are influenced by the ECM and Rho activity

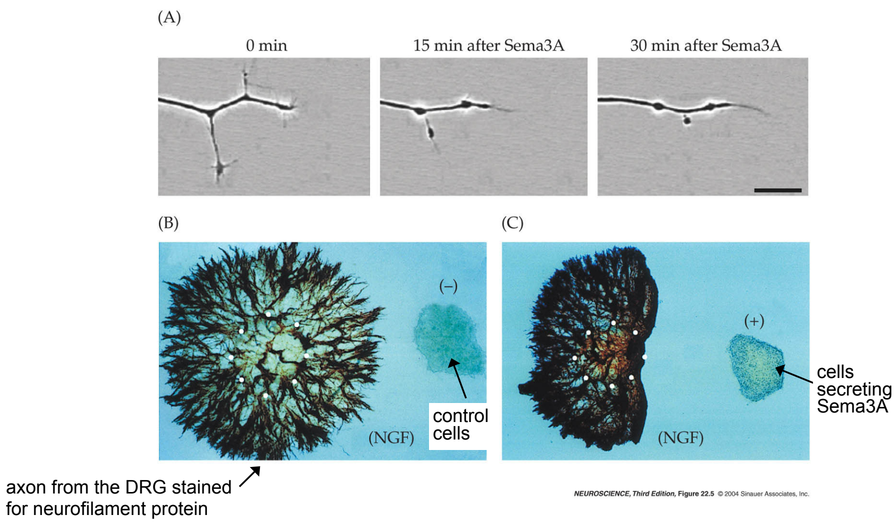




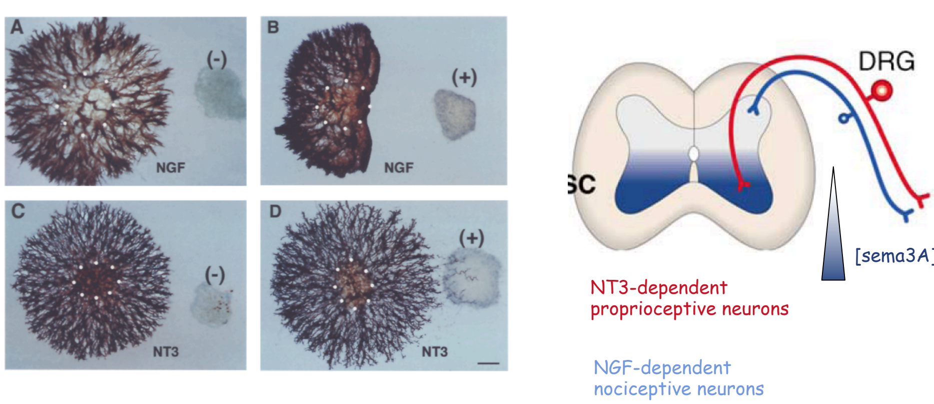
Dissociated mouse DRG from BAX-/- mice

Liu et al. (2002) Molecular and Cellular Neuroscience 20, 2–12

Semaphorin 3A is repulsive to some DRG axons...

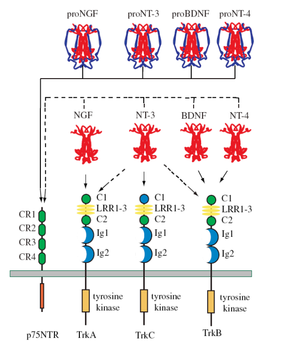


...but not other types of DRG axons

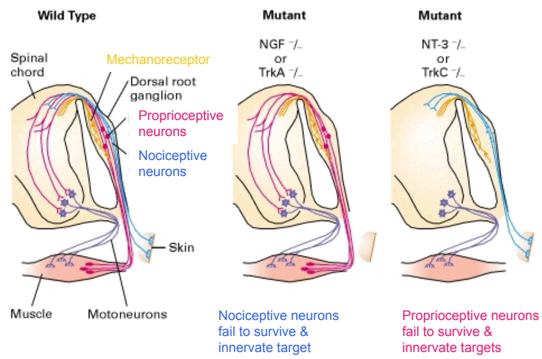


Messersmith (1995)

Different neurotrophins are important for the survival of each type of DRG neuron



Reichardt Phil. Trans. R. Soc. B (2006) 361, 1545–1564

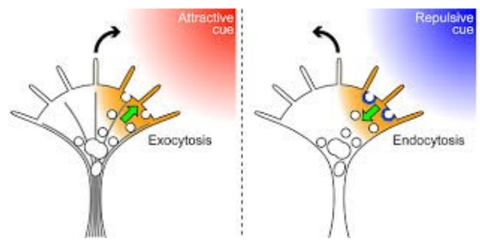


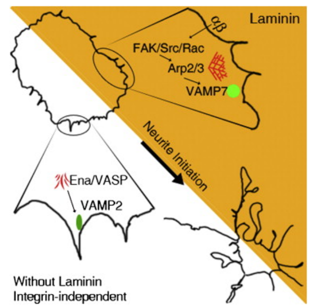
If the NT are required for survival, how can you study their role in guidance?

...block apoptosis chemically or by knockout of apoptosis genes

Response to guidance cues can change

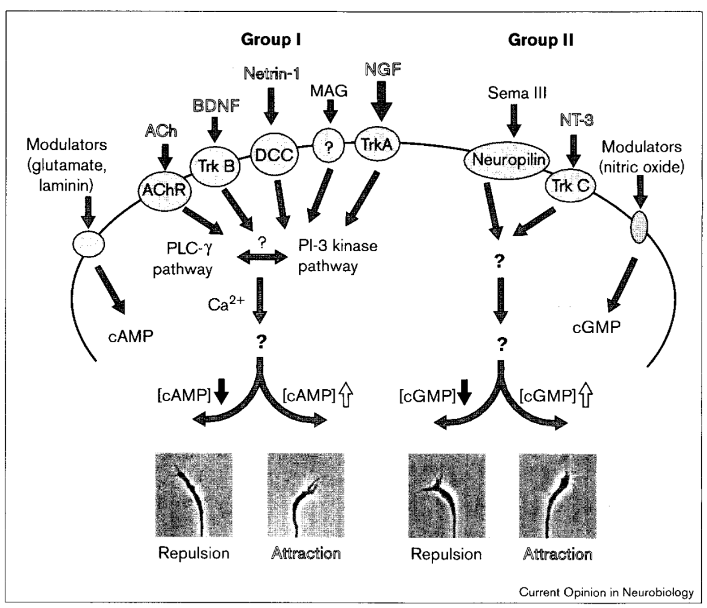
Endocytosis and exocytosis

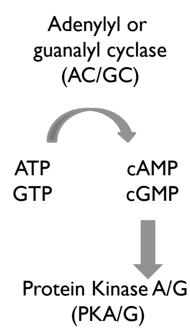




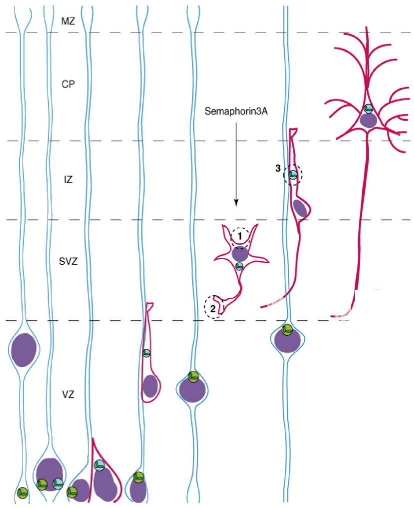
- exocytosis delivers new membrane and proteins to the expanding part of the growth cone
- endocytosis retrieves plasma membrane and membrane proteins from the retracting side
- delivery/retrieval of receptors is one mechanism of regulating signalling and adhesion
- events at the plasma membrane are coordinated with cytoskeletal activity

cyclic nucleotide effects





Perhaps, a combination of extrinsic cues and the intrinsic polarization of the neuronal perikaryon interact as immature neurons are undergoing migration to determine the location and orientation of the axon and apical dendrite.



Polleux et al (2000) Nature
Higginbotham and Gleeson (2007)

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