Adaptive signals and axon guidance

Chapter Five
Controlling direction of growth

Four types of molecular cues:

1. Contact adhesion
   - Regulated by CAMs on growth cone surface

2. Contact repulsion
   - Growth cones collapse away

3. Long range attraction
   - Diffusible molecules have positive chemotaxis to attract growth cone

4. Long range repulsion
Four sources of cues:
1. Contact Adhesion

- Regulated by CAMs
- Example of CAMs = LAMP
- Remove LAMP $\rightarrow$ abnormal directions and pathways for axons
- LAMP has three domains:
  1. Homophilic attraction
  2. Heterophilic attraction
  3. Unknown purpose
Hetero vs. homophilic

• Homophilic = proteins of the same type bind to each other
  – Interact

• Heterophilic = proteins of different types bind to each other
  – Interact
Subway lines

• Early axon tracts can be thought of as subway lines
  – Orange, red and green lines
• Each “line” expresses different CAMs on cell surface
• Newly formed axons follow path depending on which CAMs they have on their surfaces
Labeled Pathway Hypothesis: Axon Growth is Determined by The Presence of Label Axons along the Growing Pathway

The first axon that grows out (pioneer axon) generate the original tract for other axons to follow

Letourneau (1975)
CAM changing

- Expression of different molecules in different segment allow axons to fasciculate (travel together) or defasciculate (separate at critical points) according to needs

- Axons can change which CAMs they are expressing
  - Change with pathway they will take

- Like changing subway trains
Some CAM molecules mediate heterophilic interactions with other molecules. For example, Axonin-1 can bind to NCAM.

Expression of Axonin-1 allows the axon of commissural neurons to cross the midline in the chick spinal cord which express NCAM.

Antibodies against Axonin-1 or NCAM can be used to perturb their interaction.
Adhesive Substrates Does Not Direct Growth Cone!

- The presence of a permissive substrate allows axon growth...but does not direct axon projections (unless the permissive substrate is spatially restricted)

- Laminin gradient does not direct axon growth, only support axon growth beyond a minimal concentration
2. Contact Repulsion

- Chemicals can also repel axons
  - Not slow down growth
  - Actually push growth in a different direction

- Problem with contact repulsion is that the repellent must make contact with receptor attached to cell membrane
  - Therefore binding is necessary before axon will be repelled away
  - This is why diffusible repellents are more common in cell
Semaphorins Mediate Long-Term Repulsion of Growing Axons

- Semaphorins are a family of membrane-bound or soluble proteins containing the sema group.

- Semaphorins bind to the neuropilin receptors NP1 and PN2. Semaphorins can also interact with the CAM L1 and a variety of other extracellular matrix molecules.
Semaphorins Mediate Long-Term Repulsion of Growing Axons

- Semaphorins are often involved in the repulsion of axonal growth. However, allosteric modifications by sulfated proteoglycans or chondroitin sulfates may alter the ability of semaphorins to repel axons.

- In the spinal cord, the semaphorin collapsin-1 cause retraction and collapse of growing axons.

From Bandtlow & Zimmermann, 2000
Controlling Repulsion

Two possible mechanisms for regulating contact repulsion:

1. Extracellular part of receptor can be clipped off
   • Now cell will no longer respond to repulsive signal

2. Growth cone endocytoses receptor
   • Again, cell will no longer respond
Chemotaxis

- Diffusible chemicals that either attract or repel axons
- Chemotaxis is mechanism of both:
  1. Long range attraction
     - Towards source of attractant
  2. Long range repulsion
     - Away from source of repellent
Axons can be guided by diffusible molecules by a process called chemotaxis.

Chemotaxis allows an axon to recognize small differences in the concentration of a diffusible substance in order to redirect their growth.

These diffusible molecules can act as attractant or repellent of axon growth.

From Mueller, 1999
Repulsive signals prevent sensory axons from entering the ventral spinal cord.

When cultured together, DRG neurons avoid ventral cord explants in order to growth toward dorsal targets.
Repulsion

B = Ventral is repulsive but dorsal is attractive

D = Septum is repulsive

E = Both notochord and Dermomyotome are repulsive

(After Patterson and Crain, 1981; Pini, 1992; Kaynas et al., 1997)
Attraction

(After Gunderson and Barrett, 1979)
Repulsion

- Sema3A is repulsive to Doral Root Ganglia (DRG) axons
Sensitive

• Growth cones must have a sensitive way to judge small changes in concentration across local environments

• In fact – growth cones are one of the most sensitive readers of chemical gradients known in biology
  – Can sense 1 molecule different in 1000

• Send out more or less filopodia in that direction accordingly
Commisural Neuron Are Attracted to Netrin Gradient in Floor Plate

Netrin-1 expression pattern in the floorplate

In vitro assay of floorplate & Netrin-1

dorsal neural tube

floorplate

dorsal neural tube

netrin-1 COS cells

Dorsal neural tube

Dorsal commissural interneurons

Floorplate

Netrin-expressing COS cell line

Directed axon outgrowth
Netrin is a Repulsive Signal for Trochlear Motor Neurons

- Same molecule may have dual role in axonal growth: attractant or repellent

Netrin-1 inhibits Trochlear motor axon outgrowth
Netrin and Netrin Receptors in Long-Range Attraction and Repulsion

- Netrin is a long-range chemo attractant molecule released by floor plate cells.
- Obviously diffusible.
- Netrin consists of three EGF like repeats and it is related to another cell adhesion molecule laminin.

From Neuron to Brain, IV ed.
Local vs. Global

- In vivo – truly long range attractants are fairly rare
- Instead cells mostly use intermediate targets (stepping stones)
- And locally diffusible attractants
- Local environment changes 10 to 50 μm’s
- What causes changes in environment?
  - Homeobox genes 1st set up gradients
  - Then gradients change gene expression
Local cues:

(A) Tectum

Axons still grow towards missing tectum

(B) Tectum

90°

Optic axons

Neuroepithelium

(After Harris, 1989, and Taylor, 1990)
Global Guidance Molecules Can Direct Axon Growth

- The optic nerve innervates the tectum (its primary target) independently of the location of the eyes in the head. Removal of the tectum does not prevent retinal axons from projecting to the right area.

- Transplantation of an eye into various locations in the head results in appropriate projection of the optic nerve to the tectum.
Optical Pathway

• Retinal Ganglion Cells (RGCs) have a very specific pathway they follow
• From Retina (periphery) to tectum via the optical nerve
• Details while looking at picture
- RGC pathway
Axon Guidance Clues for Retinal Axon Growth

- Retinal ganglion cells express a variety of signaling molecules for axonal guidance including semaphorins, slit and CAM.

- Expression of the slit-robo complex in the optic chiasm facilitate crossing of axons into the contralateral site of the brain.

From Neuron to Brain, IV ed.
Axon Guidance Clues for Retinal Axon Growth

Axonal growth is regulated by a multitude of factors acting as attractive or repulsive signals.
Midline

Normally:
• Neurons cross the midline only once
• And then never cross back

Mutants:
• Robo mutants – cross and recross the midline numerous times
• Commissureless mutants – never cross the first time
Axons Express Different CAM on Different Segments

- Midline mutations in Drosophila result in axons that can not cross the midline (commissureless mutant) or axons that keep crossing the midline back and forth (roundabout mutant).

- Robo is an IgG transmembrane protein expressed on both sides of the midline.
Midline Pathway – Invertebrates

• Slit expressed at midline
  – Represses Robo – a surface Receptor
• Commissureless (Comm) binds up Robo and pulls it into the center of the cell
  – Robo cannot sense Slit
  – Neuron crosses midline
• After one crossing Comm releases Robo
  – Robo returns to the surface and is repelled by Slit
Midline Pathway – Vertebrate

• Netrin is expressed at the midline
  – Acts as an attractant

• Once the vertebrate neuron crosses the midline the first time

• Loses sensitivity to netrin
  – Netrin no longer works as a attractant
Adaptation

• We have just seen an example of how neurons can adapt to respond differently to the same signal
• Axons can also change gene expression
  – To respond differently to ligands by having different receptors present

Example: Netrins act repressive to axons expressing Unc5 receptors
Netrins are attractive to axons with Unc40
Adaptation

• Axons can respond differently to the same signal depending on the surrounding environment.
cAMP expression

• High cAMP promotes polymerization of cytoskeleton
  – Axon moves towards attractant

• Low cAMP promotes depolymerization of cytoskeleton
  – Axon collapses away from repulsion

• In vivo – Laminin (an ECM) reduces cAMP expression

• Aging also decreases cAMP expression
Growing

• Originally it was thought that all proteins were made in the cell body and transported to the growth cone.

• Now proven that growth cone contains:
  – mRNAs, ribosomes, and machinery to build
  – As well as degradation machinery to remove

• Growth cone is an autonomous machine
  – Capable of continually making its own proteins to respond to changing environment.
Signal Transduction

• Signals that either attract or repulse growth cone come in through receptors on cell surface
  – Which receptors are present can be changed

• Signal is transmitted through intracellular domain
  – Kinases, phosphatases, nucleotide levels, etc

• Signals regulate and change gene expression within axon
Axon Growth and Stop Signals

- How does an axon known where to stop and make a synapse?

- In the tectum, FGF acts as a stop signal for retinal ganglion axons

- Axons are also sensitive to neurotransmitters. Neurotransmitters like dopamine, serotonin prevent axons from further growth

From Neuron to Brain, IV ed.
Axons of sensory and motoneurons regenerate in the peripheral nervous system but not in CNS
Axonal Projections Following Nerve Injury

- Injury of peripheral nerves result in reinnervation of peripheral targets and restoration of axonal projections.
- Injury of centrally located targets (CNS) does not allow for reinnervation: due to the presence of inhibitory signals in CNS.

Letourneau (1975)
Oligodendrocytes and CNS myelin are not a permissive substrate for axonal growth in culture or in vivo. Release of a myelin-associated growth inhibitory protein (Schwab factor) prevents axonal regeneration in the CNS.

Oligodendrocytes generate a repulsive signal that prevents axonal growth in vitro.

Sympathetic (SN) and retinal ganglion (RGN) neurons culture over retinal glial cell substrate. Notice that neurites of SN and RGN "loop around" oligodendrocytes (red arrow).

Schwab & Caroni (1988)
The Schwab Factor

Injection of an antibody targeting the myelin-associated growth inhibitory protein (Schwab factor) allows axonal regeneration after spinal cord injury.

Brosamle et al. (2000)
Summary

Axons growth cone needs:

1. Motor
   • Dynamic cytoskeleton

2. Signals
   • ECM and diffusible gradients
   • Attract and repulse

3. Communication
   • Signal transduction
Any Questions?

Read Chapter Six