Axon Guidance

Paul Letourneau
letou001@umn.edu

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

- 100 billion neurons
- 100 trillion synapses
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.

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

15+ years

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

1 mm
3 \times 10^2 neurons
7 \times 10^3 synapses
3.5 days

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

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

(After Wilson et al., 1996; Ross et al., 1992)
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
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.

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

Dorsal root ganglia
Control 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 LMCI 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

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**

- **Wild type Netrin expression**
  - Netrin-A
  - Netrin-B
  - 1, 2
  - 5, 8

- **Netrin loss-of-function**
  - DI(1)NP5 = (NetA" NetB")
  - 21, 22, 23

- **Ectopic Netrin B expression**
  - (in wild type background)
  - in muscle

- **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.
Comm mutant
Cross midline

Commissureless
Don't cross

Comm mutant

Robo mutant

Roundabout
Re-cross
• 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.
Comm mutant
Always express Robo

Normal
Cross midline

Commissureless
Don’t cross

Comm mutant

Always express Robo

Robo mutant

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

<table>
<thead>
<tr>
<th>Guidance cue/receptor</th>
<th>dl1 commissural axons</th>
<th>Thalamo-cortical axons</th>
<th>Corpus callosum</th>
<th>Optic chiasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netrin/Dcc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Slit/Robo</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sema3/neuropilin</td>
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<td>Sema6/plexin</td>
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<td>Ephrin/Eph</td>
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<td>Wnt/Frz3 or Ryk</td>
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<tr>
<td>Draxin/Dcc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
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</tbody>
</table>
• 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.
Guidance cues act through a series of steps to regulate growth cone behaviors.
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

A. Expose growth cone to a graded concentration of cytochalasin to depolymerize actin on one side.

Local microtubule depolymerization
Turns growth cone away from pipette

C. Destabilize microtubules on one side with nocodazole.

Local microtubule stabilization
Turns growth cone towards pipette

B. Stabilize microtubules on one side with taxol.
Growth cone-substrate adhesions
Migrating growth cones gain adhesion to their substrate by way of several types of adhesion molecules.
Integrin dimer = adhesion receptor

Talin = actin-integrin linker

Vinculin = actin-integrin linker

Alpha-actinin = F-actin linker

Integrin dimer = adhesion receptor

ABPs link F-actin bundles to adhesive sites

Substratum
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.
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.
Attractive cues

Repulsive cues

Microtubules swept back by retrograde actin flow

filopodia initiated via cdc42 and rac

microfilaments

dynamic microtubules

stable microtubules

microtubule capture and stabilisation via TIPs and MAPs

membrane vesicles routed along stable microtubules

collapse of actin cytoskeleton via rho

rho activity

cdc42 and rac activity
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

ATP
  \[\text{adenylyl cyclase}\]
  cAMP

GTP
  \[\text{guanylyl cyclase}\]
  cGMP

Protein effectors

PKA
  other effectors
  Including
  ion channels, VASP (an ABP), CREB, GTPases, phosphoproteins

other effectors

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

Ming et al (1997), Neuron
Repulsion from semaphorin 3A is switched to attraction by enhancing cGMP signaling.

Repulsion

Repulsion

Repulsion switched to attraction

Song et al (1998), Science
Guidance Cue

Receptor

Signal  cAMP, cGMP  [Ca++]  Nitric oxide

Target

Cytoskeleton

Growth Cone Behavior

Diagram:
- Attractive cues
- Repulsive cues
- Rac-GTP
- Cdc42-GTP
- RhoA-GTP
- ROCK
- N-WASP
- Arp2/3
- Coffin
- CRMP
- MLCK
- PAK1
- LIMK
- RLC
- Nucleates myosin II

Output:
- Decreased retrograde actin flow
- Increased actin nucleation
- Increased actin dynamics
- ?

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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)
- \(\text{calcineurin}\) (phosphatase)
- gelsolin (breaks actin filaments)
- \(\alpha\)-actinin (bundles actin filaments)
- myosin II (motor protein)
Attractive Ca\(^{2+}\) signal

- VAMP2-mediated exocytosis
- Attraction

Repulsive Ca\(^{2+}\) 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 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++]
  ↓
Targets Nitric oxide, Protein synthesis, degradation
  ↓
Cytoskeleton
  ↓
Growth Cone Behavior
Growth cones must integrate signaling simultaneously triggered by multiple guidance cues.