Axon guidance II

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March 29, 2004
Last time: axon guidance cues/receptors

- Axons are guided to target region by extracellular guidance cues which interact with axonal receptors
- Guidance cues can be diffusible or membrane-bound, act locally or over a distance
- A given guidance cue can trigger attraction or repulsion --- depends on:
  - Repertoire of guidance receptors
  - Others cues received
  - State of signaling pathways in growth cone
Reaching the target region

• Axons usually pass through intermediate targets to reach final target
• Navigational decisions involve the integration of multiple signals in an axon-specific fashion:
  – Example: regulation of projection to and across the midline via Netrin, Slit, Robos and Comm
Major navigational forces

- Semaphorins (secreted)
- Netrins
- Long-range cues
- Netrins
- Chemoattraction
- Growth cone
- Contact repulsion
- Eph ligands
- Semaphorins (transmembrane)
- ECM (for example, tenascins)
- Short-range cues
- Ig CAMs
- Cadherins
- ECM (for example, laminins)

http://www.science.gov/content/vol274/issue5290/images/large/se466435501.jpg
Navigation in 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
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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
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Eph and Ephrins in the Retinotectal System

- Expressed in complementary gradients on axons/targets.
Topographic maps

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Mammalian olfaction

- **Mice:** \( \approx 1500 \) olfactory receptor genes (7-TM receptors)
- **Humans:** \( \approx 1000 \) olfactory receptors (\( \approx 2/3 \) pseudo-genes)
Olfactory receptor gene expression

• 1 Olfactory receptor per olfactory neuron
• Diploid: 2 alleles of each olfactory receptor locus --- only 1 of the 2 loci is expressed (monoallelic expression)
• Analogous to expression of B and T cell receptors -- single B or T cell receptor expressed via DNA rearrangement
• Recent work suggests olfactory receptor expression does not involve irreversible alterations to DNA:
  Take nucleus from differentiated olfactory neuron and use to clone a whole mouse --- this mouse has apparently normal diversity of OR expression
• How an individual OR expresses only a single allele of a single OR is unknown
Wiring of the Olfactory System

- Over a thousand different types of olfactory neurons (each type expresses a particular receptor)
- Problem of odor distinction requires determining which olfactory neurons are activated
- How tell which olfactory neurons are activated?
Olfactory axons expressing the same receptor project to the same target in the olfactory bulb.
Olfactory receptor expression is not entirely random: epithelium segregated into zones
Targeting of olfactory axons depends on olfactory receptor expression
Olfactory axon target specificity depends on identity of olfactory receptor
Olfactory axon map formation

- Target selection specificity involves olfactory receptor
- Role of olfactory receptor unclear:
  - traditional guidance receptor? if so, what are the cues?
  - activity-dependent mechanisms enforcing specificity? “fire together, wire together”
- Other cues may interact to help establish zonal projection pattern (Eph/ephrin gradients)
- Similar kind of convergence observed in fly olfactory system -- ≈50 glomeruli; receptor-independent
Cell-cell recognition molecules implicated in target cell selection in the retina

- **Sidekicks**: Transmembrane members of the Immunoglobulin superfamily (IgSF) : 6 lgs, 13 Fns
- **Chickens** have two Sidekicks: Sdk-1, Sdk-2 (59% identical)
Sidekicks (Sdks) are homophilic cell adhesion molecules (CAMs)

- Sdk’s act as homophilic cell adhesion molecules
  - Homophilic: interact with the same protein on adjacent cell
  - Heterophilic: interact with different protein on adjacent cell
- Sdk1 binds Sdk1
- Sdk2 binds Sdk2
- Sdk1 and Sdk2 do not associate
Sidekicks in axon/dendrite recognition in the chick retina

- Sdk-1 and Sdk-2 are expressed by distinct subsets of Retinal Ganglion Cells (RGCs) (each in ≈25% of RGCs) and presynaptic inputs of RGCs (amacrine cells, bipolar cells).
- Evidence suggests:
  - Sdk-1⁺ amacrine/bipolar axons contact Sdk-1⁺ RGC dendrites
  - Sdk-2⁺ amacrine/bipolar axons contact Sdk-2⁺ RGC dendrites
- Sdks could allow specific subsets of axons and targets to recognize one another
Sidekicks can alter axon/target specificity

- Express Sdk-1 in Sdk-negative amacrine cells
  - axons retarget to the Sdk1⁺-layer (figure)
- Express Sdk-1 in Sdk-negative RGCs
  - dendrites retarget to the Sdk1⁺-layer
- Also true for Sdk-2
- Thus, Sdk expression can regulate target selection
Sidekicks in target specificity

- Differential Sdk expression may underlie differential target recognition in the retina (no loss-of-function yet)
- Sdks localize to synaptic sites --- could assist assembly of pre/post-synaptic sites
- Sdks also have intracellular domains --- Sdks likely act not only through surface adhesion, but by coupling to cytoskeleton and intracellular signaling pathways
Capricious: a transmembrane protein important for target selection

- Doesn’t act as homophilic CAM.
- Expressed on ~1/3 of *Drosophila* body wall muscles.
- Expressed on axons that innervate these muscles.
Capricious: target selection molecule in *Drosophila* motor axon/muscle system

- Necessary and sufficient for target selection (sort of..)
- Effects are partial
- Expressed on a large fraction of neurons and targets

Probably part of a combinatorial code.
Collateral branching and selective retention: Axon targeting can involve remodeling

- Layer 5 neurons initially contact multiple target regions
- Neurons from different areas of cortex contact similar target regions
- Final projection patterns established through selective elimination of axon segments
Dendrite guidance

- A neuron’s axons and dendrites project to different targets
- How can different projections from a single cell choose distinct targets?
- Do different cues/receptors guide dendrites?
  - No -- axon guidance cues/receptors also guide dendrites
- How the same cue affect axons and dendrites differently?
A neuron’s axons and dendrites can respond differently to the same signal

- Cortical neurons respond to the secreted cue Sema3A
- Sema3A is present in a graded fashion (highest apically)
- Axons and dendrites both respond to Sema3A, but differently
  - Axons repelled, dendrites attracted
How do the axons and dendrites respond differently to Sema3A?

- Different receptors? No
- How generate different responses in same cell?
- Recall: cyclic nucleotide levels and additional cues can switch a growth cone’s response to a cue (Netrin, cAMP, laminin example)
- Sema3A response is sensitive to cGMP levels in *in vitro* turning assays:
  - High levels of cGMP: Sema3A is attractive
  - Low levels of cGMP: Sema3A is repulsive
Nature of response to Sema3A affected by cGMP signaling

- Soluble guanylyl cyclase (sGC) produces cGMP
- sGC is concentrated in cell body and dendrite; not present in axon
- Suggests:
  - higher levels of cGMP in dendrite (attraction)
  - lower levels of cGMP in axon (repulsion)
Nature of response to Sema3A affected by cGMP signaling

- Inhibit sGC or Protein Kinase G (cGMP-regulated kinase):
  - No effect on axon
  - Apical dendrites grow randomly
- Consistent with:
  - High cGMP dendrite --- attracted by Sema3A
  - Low cGMP axon --- repelled by Sema3A
- Repertoire of downstream signaling molecules appears responsible for differential response to Sema3A
The growth cone

- Structure at leading edge responsible for navigation

- Responds to cues within minutes.

- Can continue to navigate (for a while...) if severed from cell body.
The growth cone cytoskeleton

- microtubules
- filopodia
- F-actin

guidepost cell
Actin
Microtubules

(A) β-tubulin and α-tubulin

(B) Tubulin heterodimer (α = microtubule subunit)

(C) Protifilament

(D) Microtubule

(E) TEM image of microtubule
Cytoskeletal changes underlie cell movements

(A) small soluble subunits $\rightarrow$ large filamentous polymer

(B) signal, such as a nutrient source:
- Disassembly of filaments and rapid diffusion of subunits
- Reassembly of filaments at a new site
The growth cone cytoskeleton is both highly structured and dynamic
Actin dynamics suggest two levels for the control of growth cone motility by signals

1. Filament assembly/disassembly
   - polymerization/depolymerization
   - filament nucleation and filament capping
Actin dynamics suggest two levels for the control of growth cone motility by signals

2. Retrograde flow
   - myosin motor driven
   - counteracted by coupling to substrate?
Microtubule dynamics
Microtubule organization is also highly dynamic and regulated.
How do guidance receptors regulate cytoskeletal structure?

- Rho-family GTPases are important targets for relaying information from receptors to the actin cytoskeleton.


  injected activated Rho GTPases into fibroblasts
  --got dramatic reorganization of actin cytoskeleton within minutes!!
Injection of activated GTPases into fibroblasts

- activated Rho: stress fibers at focal adhesions
- activated Rac: actin meshwork at leading edge
- activated cdc42: actin bundles

• Rho family GTPases are powerful regulators of the actin cytoskeleton.
• Different family members can have different effects
RhoGTPases influence actin dynamics at many different steps

- Actin dynamics:
  - nucleation of new actin filaments
  - polymerization/depolymerization of existing filaments (capping/uncapping of filament ends)
  - retrograde flow of actin filaments (myosin-dependent)
Rho GTPases work through effectors

• Rho GTPases regulate protein and lipid kinases, scaffolding proteins…
• Different Rho GTPases cause different changes in cell structure and movement because they regulate different sets of effectors
Possible routes from Rho-family GTPases to the cytoskeleton

<table>
<thead>
<tr>
<th>Route</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Rac → Pak</td>
<td>Inhibit retrograde flow</td>
</tr>
<tr>
<td>Rac → PI4,5 kinase → PIP2 → Capping Protein</td>
<td>Uncap actin filaments</td>
</tr>
<tr>
<td>Cdc42 → N-Wasp → Arp2/3</td>
<td>Nucleate new actin filaments</td>
</tr>
</tbody>
</table>
Rho family GTPases act as molecular switches

- members of the ras superfamily of p21 GTPases
- subject to both positive and negative regulation by:
  - guanine nucleotide exchange factors (GEFs) (positive)
  - GTPase activating proteins (GAPs) (negative)
The p21 GTPase cycle
How can putting a Rho-family GTPase into the GTP-bound regulate an effector?

One example: **PAK** (a serine/threonine protein kinase) is activated by binding to \( \text{Rac}^{\text{GTP}} \) and \( \text{cdc42}^{\text{GTP}} \).

Crystallographic and biochemical data suggest this type of activation mechanism may be used in many RhoGTPase-effector interactions.
Rho GTPases in neural development

- Mutations in Rho-family members disrupt axon guidance (and other aspects of neuronal morphology)
- Mutations in Rho-family regulators and effectors are found in a number of mental retardation syndromes in humans
  - MRX46: Rac/Cdc42 GEF
  - MRX 60: Rho-family GAP
  - MRX30, MRX47: Pak3 (mutations in GBD)
Rho family GTPases are important targets of axon guidance receptors

- Robo signals through a GAP for cdc42 called srGAP
- Slit/Robo response blocked by interfering with srGAP function or using a mutant form of cdc42 locked into the GTP state

- Consistent with this type of pathway for Slit/Robo repulsion:

  Slit $\rightarrow$ Robo $\rightarrow$ srGAP $\rightarrow$ cdc42 $\rightarrow$ Filapodial extension
Rho family GTPases are important targets of axon guidance receptors

- The PlexB guidance receptor for Semaphorin family cues binds directly to Rho GTPases
- Sema binding to PlexB stimulates signaling by Rho and inhibits signaling by Rac (prevents binding to Pak)
- Consistent with this type of pathway for Sema/PlexB repulsion:

Sema → PlexB → Rho → Retraction

activates

Rac → Pak → Lamellapodial extension

inhibits
Guidance receptor signaling to the cytoskeleton

• Guidance receptors interact with Rho GTPases and their regulators to generate the cytoskeletal changes responsible for guidance

• However: Rho GTPases are not the whole story: there are additional routes from receptors to the cytoskeleton
  – For example: Mena proteins bind to guidance receptors and directly to actin filaments
Responding to chemotropic gradients

- Axons need to be able to sense and grow toward or away from sources of diffusible cues
- How sense and response to potentially shallow gradients of cues?
- Detailed molecular mechanisms in growth cone not yet known
  - Appears to involve repeated cycles of sensitization/desensitization (axons zig-zag)
- Molecular mechanisms beginning to be worked out in leukocytes and in Dictyostelium discoideum (slime mold)
Visualizing cytoskeletal changes in Dicty cells sensing chemoattractant

[GFP-Actin: cells chemotaxing towards cAMP from a pipette.](http://dictybase.org/tutorial/gerisch1.avi)

- The tip of the pipette is moved as indicated.
- Images were captured every 18 seconds.

- From K. Barisic, M. Ecke, C. Heizer, M. Maniak, M. Westphal, R. Albrecht, G. Gerisch, Max-Planck-Institut fur Biochemie, Martinsried, Germany.
Visualizing the activity of signal transducers in Dicty cells

- Can look at changes in subcellular distribution of key regulators of chemotaxis
Role of phospholipid signaling in Dictyostelium chemotaxis

- Phosphorylation of membrane lipids can serve as a localized signal to activate downstream signaling proteins
  - For example: Phosphorylation of PIP2 can activate PH domain containing proteins like the kinase Akt
Two regulators of phospholipid signaling are essential for chemotaxis

- PI3-Kinase: lipid kinase
- PTEN: lipid phosphatase
- PI3-K and PTEN antagonize one another
- Both are important for robust chemotaxis
Visualizing the dynamics of PIP3 production of PIP3 in Dictyostelium

http://dictybase.org/tutorial/VIDEO1.AVI

• Translocation from to the plasma membrane of GFP-tagged PH domain in response to a uniform increase in chemoattractant.
• Monitors production of PIP3 by PI3-kinase (lipid kinase)
• See transient increase in PIP3 at membrane
• Frames were taken every 2 seconds.
PI3-K and PTEN alternate at the membrane
Model of PIP3 production

- Alternating activation/inhibition of PIP3 production

Unpolarized cell exposed to uniform stimulus:

- t=0 sec
- PTEN binding site
- PI3K binding site
- Actin polymerization

- t=5 sec
- PTEN
- PI3K
- Actin polymerization

- t=180 sec
- cAMP
- Excitation
- Inhibition
Visualizing the enzymes responsible for controlling PIP3

- See increase in PI3K at leading edge
- See increase in PTEN at trailing edge

Source of chemoattractant
Current models of response of Dictyostelium cells to chemoattractant

Polarized cell exposed to a gradient:

Gradient