Hierarchical Organization of Guidance Receptors: Silencing of Netrin Attraction by Slit Through a Robo/DCC Receptor Complex

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*Science* **291** (March 9, 2001)

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Growth cone

- Motile structure leading axon extension
- Directs neuron to target

http://www.cbit.uchc.edu/faculty_nv/lesfigs5.html
Growth cone guidance

Receptors

- **DCC (Deleted in Colorectal Cancer)**
  - Expression is reduced in colorectal and other cancers (tumor suppressor?)
  - Required for netrin-1-mediated axon outgrowth

- **Robo (Roundabout)**
  - Repulsive guidance receptor
  - Binds to Slit in the midline
1. **Attraction to midline:**
   netrin activation of DCC

2. **Crossing and moving from the midline:**
   - Event 1: Upregulation of Robo expression,
     repulsion by Slit
   - Event 2: Loss of netrin responsiveness,
     despite maintained DCC expression

Figure 1
Mutants

T. Kidd, K.S. Bland, C.S. Goodman,
*Cell* **96** (1999)
Turning angle

- *Xenopus* spinal axons in culture
- Gradients of soluble factors

Figure 2A
Silencing of netrin attraction

- Attracted by netrin-1
- Attracted by BDNF
- Slit2+netrin-1 $\Rightarrow$ no attraction, but still growth
- Slit2+BDNF $\Rightarrow$ no silencing
- What’s unexpected here?

Figure 2B
Slit2 Repulsion

Figure 2D, 2E
Mechanisms for silencing?

Model 1: Ligand-Ligand interaction

Model 2: Receptor-Mediated Silencing

Figure 3A
Truncated rRobo1

Does this rule out the first mechanism?
Met-DCC Chimeric Receptor

Figure 3C

does HGF

DCC cytoplasmic domain

Met cytoplasmic domain

Figure 3C
Met-Robo1 and trkA-Robo1

Figure 3D
Cytoplasmic domains are required
Robo1 receptor (or chimera with Robo1 cytoplasmic domain) must be activated
Different ligands (as long as they match the receptors) have the same result
Activated rRobo1 will associate to myr-DCC
Myristoylated cytoplasmic domains associate
  - Full-length proteins repress association
  - Slit2 derepresses the association
Yeast two-hybrid analysis

Loss of ability to rescue growth on histidine-deficient plates

Figure 4G, 4H
Met-RoboΔCC1

- What happens to attraction/repulsion/silencing?
Met-RoboΔCC1

Figure 5B
Met-DCCΔP3

Figure 5D
Met-DCCΔP3-SAM

Restored attraction, no silencing

Figure 5F
trkA-Robo1 and Met-DCC

Attraction, no silencing

trkA cytoplasmic domain

Figure 6A, 6B
Restoration of silencing

Figure 6C, 6D
Model Summary

**Stage 22**
- **Attraction**
  - Growth stimulation
- **Silencing**
  - Growth stimulation

**Stage 28**
- **Repulsion**
  - Growth stimulation

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Figure 7
Discussion

- Up-regulation of Robo receptors?
- What happens between st. 22 and st. 28 to cause Slit to repel the growth cone?
- Are DCC family receptors elsewhere that do not respond to netrin being silenced similarly?