Directional guidance of neuronal migration

- progress has been made in the last few years on mechanisms guiding the direction of neuronal migration
- it is now clear that *migrating neurons* are guided by molecular cues that also guide the *projection of axons*
Several molecular linkages between receptors for neuronal guidance cues and Rho GTPases have been recently analyzed in vitro and in vivo. srGAP is a robo-binding GAP protein that principally inactivates cdc42. Ephexin is a Eph-binding GEF that mediates ephrin-induced RhoA activation. RhoA and Rac are known to directly bind to a semaphorin receptor, Plexin. The molecular linkage between netrin receptors and RhoGTPases has not been identified yet even though netrin regulates Rho GTPase activity in a heterologous system.

**Table 1.** Directional Guidance cues involved in CNS neuronal migration in vivo and in vitro

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Receptors</th>
<th>Defects in CNS neuronal migration in vivo</th>
<th>Neuronal migration in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slits</td>
<td>Robo</td>
<td>—</td>
<td>1. Slit repels postnatal SVZ cells[123]</td>
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<tr>
<td>Netrins</td>
<td>DCC</td>
<td>1. Abnormal pontine nuclei in DCC and netrin-1 receptor[125]; 2. Slit repels prefrontal SVZ cells of GABAergic neurons[126]</td>
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<td></td>
<td>Unc-5h</td>
<td>2. Abnormal cerebellar development in unc-5h[124]; 1. Netrin-1 attracts pontine axons[177]</td>
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<tr>
<td>Semaphorins</td>
<td>Neurexin</td>
<td>1. Abnormal GABAergic neurones in the stratum in neurexin-2 mutants[129]; 3. Anti-DCC antibody blocks directed migration of postnatal SVZ cells[130]</td>
<td></td>
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<tr>
<td>Ephrins</td>
<td>Eph</td>
<td>1. Disruption of Eph-B/Ephrin-B system affects the migration of postnatal SVZ cells[131]</td>
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*Unc-5h* [ROM mutant mice showed abnormal development of cerebellum. However, it is still unclear that the defect is primarily caused by migration abnormality or other reasons.

**Rho GTPases signaling mechanisms of neuronal and leukocyte guidance cues.**

DCC, Deleted in Colorectal Cancer; Robo, Roundabout; G, Heterotrimeric G proteins; Ig, Immunoglobulin domains; FNIII, Fibronectin type III repeats; TS, thrombospondin type 1 repeat; Glb, globular; Cys, Cysteine-rich; Sema, Semaphorin; MRS, Met-related sequence; G-P, Glycine-proline repeat; CUB, Complement binding; FV/VIII, Coagulation factor V/VIII homology; MAM, Meprin.
**Directional Guidance cues involved in CNS neuronal migration in vivo and in vitro**

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<td>1. Slit repels postnatal SVZa cells^{28}</td>
</tr>
</tbody>
</table>
| Netrin  | DCC       | 1. Abnormal pontine nuclei in DCC and netrin-1
          | Unc-5h    |                                           | 2. Slit repels prenatal SVZa cells of GE^{23} |
|         |           | 2. Abnormal cerebellar development in unc-5h^{45} | 1. Netrin-1 attracts pontine nuclei^{17} |
| Semaphorins | Neurophin | 1. Abnormal GABAergic terminations in the striatum in
          | Eph       |                                          | 2. Netrin-1 repels postnatal cerebellar granule
          |           |                                          | cells and prenatal SVZa cells^{49,48} |
|         |           |                                          | 3. Anti-DCC antibody blocks directed migration of
          |           |                                          | postnatal SVZa cells^{49} |
|         |           |                                          | 1. Disruption of Eph-B/Ephrin-B system affects
          |           |                                          | the migration of postnatal SVZa cells^{50} |

^{*}Unc-5h/ROM mutant mice showed abnormal development of cerebellum. However, it is still unclear that the defect is primarily caused by migration abnormality or other reasons.

**Eph receptors and ephrin ligands**

Binding specificities of Eph receptors and ephrins. Eph receptors and ephrins fall largely into two binding specificity classes, with the exception of EphA4, which interacts with ephrin-A and some ephrin-B proteins. Differences exist, however, in the relative affinity of a receptor for different ephrins that may be functionally important. Additional ephrins probably exist, because EphB5 does not bind to any known ephrin.

*glycosylphosphatidylinositol membrane anchored*
In animals with binocular vision, most retinal axons (red) cross to the contralateral side of the brain, while a smaller subset of retinal axons (blue) project to the ipsilateral side. Retinal axons expressing EphB1 are repelled from the optic chiasm by ephrinB2 and directed to an ipsilateral pathway. Contralaterally projecting axons do not express EphB receptors and therefore are not repelled by ephrinB2.
Eph receptors and ephrins restrict cell intermingling and communication

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- assay in which an Eph receptor and an ephrin are expressed in adjacent cell populations and the amount of cell intermingling determined
- Zebrafish embryos at the one-cell stage are injected with fluorescent lineage tracer and then animal caps are dissected at the 1,000-cell stage
- animal caps that were labelled with rhodamine dextran (LRD) or fluorescein dextran (LFD) were juxtaposed and co-injected with RNA encoding Eph receptor or ephrin-B, respectively
- after overnight culture, serial confocal sections of the fluorescent tracers were visualized

The zebrafish (Brachydanio rerio) is a small aquarium fish used as a model system for vertebrate developmental biology.

rhodamine dextran (LRD) + Eph receptor mRNA (20-100pg)
fluorescein dextran (LFD) + ephrin ligand mRNA (20-100pg)

1 cell stage (10' after fertilization)
Scale bar: 250 µm.

1000-cell stage (3 h after fertilization)
Bidirectional but not unidirectional signalling restricts cell intermingling

Quantification of cell intermingling.

The bars indicate the average number of isolated single cells appearing in the adjacent territory for the indicated combinations of Eph receptors in one animal cap (below the line) and ephrin in the other (above the line). N, number of aggregates analysed. Intermingling is significantly reduced compared with uninjected controls (P, 0.01; indicated by asterisks) in combinations that give bidirectional activation of Eph receptor and ephrin-B. More intermingling compared with uninjected controls occurs (P, 0.01) when, for example, one cell population expresses full-length Eph receptor, and the other truncated ephrin. This could result from some autoactivation of full length Eph receptor or ephrin-B that decreases cell-cell adhesion and thus increases the amount of cell movement.
Might Eph receptors regulate the formation of gap junctions?

- Gap junctions form by assembly of connexin proteins into intercellular channels that allow passage of molecules with a relative molecular mass below 1.2KDa and can be detected by the ability of Lucifer Yellow to diffuse through these channels.
- Juxtaposition of one animal cap labelled with **Lucifer Yellow** (green in the confocal image), and another labelled with **rhodamine dextran** (red fluorescence).
- When gap junctions have formed between the cell populations, Lucifer Yellow transfers into rhodamine dextran-labelled cells and the overlap leads to a **yellow** signal.

Activation of Eph receptor or ephrin blocks communication through gap junctions.

- When gap junctions have formed between the cell populations, Lucifer Yellow transfers into rhodamine dextran-labelled cells and the overlap leads to a **yellow** signal.
- When EphA4 or EphB2 were expressed in one animal cap and ephrin-B2 in the other, Lucifer Yellow did not diffuse between the cell populations.
- After unidirectional activation of ephrin-B2 by truncated EphB2 or of EphB2 by truncated ephrin-B2, Lucifer Yellow did not transfer into rhodamine-labelled cells, despite intermingling of the cell populations.
- These results indicate that unidirectional signalling is sufficient to block the formation of gap junctions.
General features of ephrins and Ephs and the concept of bi-directional signaling

Schematic diagram depicting an ephrin-expressing cell (top) in contact with an Eph-expressing cell (bottom).

The globular domain is the ephrin-binding site of the Eph receptor. Ephrin–Eph interactions can trigger reverse signaling into the ephrin-expressing cell, forward signaling into the Eph-expressing cell, or bi-directional signaling into both cells.