Slit proteins: molecular guidance cues for cells ranging from neurons to leukocytes

Kit Wong, Hwan Tae Park*, Jane Y Wu* and Yi Rao†

Recent studies of molecular guidance cues including the Slit family of secreted proteins have provided new insights into the mechanisms of cell migration. Initially discovered in the nervous system, Slit functions through its receptor, Roundabout, and an intracellular signal transduction pathway that includes the Abelson kinase, the Enabled protein, GTPase activating proteins and the Rho family of small GTPases. Interestingly, Slit also appears to use Roundabout to control leukocyte chemotaxis, which occurs in contexts different from neuronal migration, suggesting a fundamental conservation of mechanisms guiding the migration of distinct types of somatic cells.

Addresses
Department of Anatomy and Neurobiology, and *Departments of Pediatrics and Molecular Biology and Pharmacology, Box 8108, Washington University School of Medicine, 660 S Euclid Avenue St Louis, Missouri 63110, USA
*e-mail: jwu@pcg.wustl.edu
†e-mail: raoyi@thalamus.wustl.edu

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Abbreviations

Abl	Abelson
DCC	Deleted in Colorectal Cancer
DRG	dorsal root ganglion
EGF	epithelial growth factor
Ena	Enabled
GAP	GTPase-activating protein
GE	ganglionic eminence
HEK	human embryonic kidney
Ig	immunoglobulin
LRR	leucine-rich repeat
RGC	retinal ganglion cell
Robo	Roundabout
SDF-1	stromal-derived factor-1
SVZa	subventricular zone

Introduction

How migrating cells are guided to their destination is a question of general importance in cell biology. Recent studies initially focused on the guidance of axons, a specialized neuronal process, have been extended to other cell types and led to conclusions of broad interests. In this review, we follow the studies on the Slit family of proteins and discuss how they provide a molecular perspective that leads to conclusions of general interests. Mutations in *slit* were uncovered in *Drosophila* by Nüsslein-Volhard, Wieschaus and Kluding in their screen for genes involved in pattern formation [1]. The cDNA for *Drosophila slit* was isolated by Rothberg and Artavanis-Tsakonas from a molecular screen based on partial homology to the epithelial growth factor (EGF) repeats of the Notch protein [2]. *slit* mutants exhibit midline defects; the differentiation of the midline glial cells was thought to be abnormal [2,3]. Projection of the commissural axons was also abnormal: instead of crossing the midline once before projecting longitudinally, the commissural axons from two sides of the nerve cord are fused at the midline in *slit* mutants [2,3]. Because the midline glial cells are known to be important in axon guidance, the commissural axon phenotype in *slit* mutants was initially thought to be secondary to the cell-differentiation phenotype [3].

In early 1999, results from three groups demonstrated independently that Slit functioned as an extracellular cue to guide axon pathfinding [4–6], to promote axon branching [7], and to control neuronal migration [8]. The functional roles of Slit in axon guidance and neuronal migration were soon supported by other studies in *Drosophila* [9] and in vertebrates [10–14]. The family of Slit proteins has now been found in multiple species and functional studies have progressed significantly in the past few years.

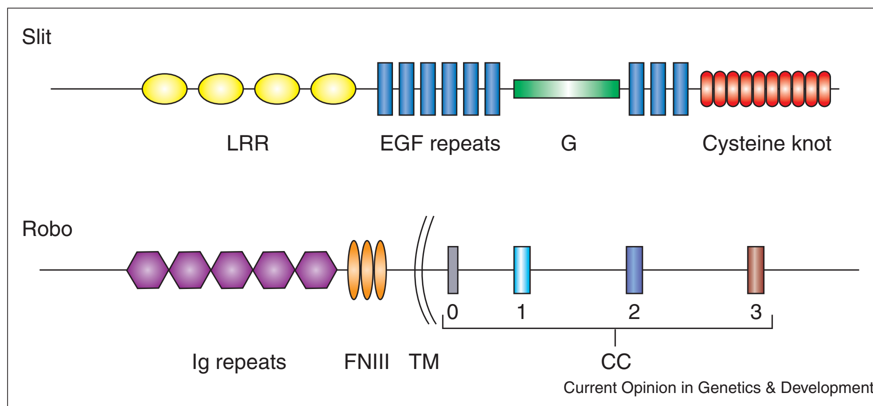
The Slit proteins

Slit genes have been found in *Drosophila*, *Caenorhabditis elegans* [15•], *Xenopus* [6,14], chickens [6,16], mice [4,6,17], rats [18,19•] and humans [6,18]. In mammals, the Slit family consists of three members that are all expressed in the ventral midline (the floor plate) of the neural tube. Slit-1 is predominantly expressed in the nervous system whereas Slit-2 and -3 are also expressed outside the nervous system [13,17,18,19•,20]. Mammalian Slit expression continues into adulthood [19•,21••], suggesting roles in addition to those during development.

A prototypical Slit protein contains an N-terminal signal peptide, four leucine-rich repeats (LRRs), seven (in *Drosophila* Slit) or nine (in vertebrate Slits) EGF repeats, and a C-terminal cysteine knot [2,3,22] (Figure 1). The LRRs are sufficient for Slit interaction with the receptor [23•,24•]. Several Slit isoforms are created by post-translational modification. Proteolytic processing of the human Slit-2 protein gives rise to an N-terminal fragment (Slit-N) and a corresponding C-terminal Slit fragment (Slit-C) [4,7]. Slit-N contains all four LRRs and five of the EGF repeats (amino acids 1–1117) whereas Slit-C contains the rest of the protein [7]. Both the full-length and the fragments of Slit are secreted extracellularly [2,4,6,7], although Slit-N appears to be more tightly associated with the cell membrane [7]. Mammalian Slit-3 protein has been reported to localize in the mitochondria [25], although the significance of this observation is unknown.

There are functional differences among the full-length and the fragments of Slit. Both the full-length Slit and Slit-N

Figure 1



Primary structures of mammalian Slit and Robo proteins. The mammalian Slit protein contains four LRRs, nine EGF repeats, a laminin G domain and a cysteine rich C terminus. *Drosophila* Slit lacks the eighth and ninth EGF repeats. A prototypical Robo receptor contains five Ig repeats, three fibronectin (FN) type III repeats, a transmembrane domain (TM) and four conserved cytoplasmic (CC) motifs. *Drosophila* Robo and mammalian Robos contain all four cytoplasmic motifs whereas *Drosophila* Robo2 and 3 do not contain some of the CC domains.

can repel axons and neurons [23•,24•,26•]. Although both the full-length Slit and Slit-N could collapse the growth cones of retinal ganglion cells (RGCs), it was reported that only Slit-N could collapse the growth cones of olfactory bulb axons [26•]. Slit-N can promote the branching of axons from the dorsal root ganglion (DRG), whereas the full-length Slit antagonizes this activity [7,26•].

Although the sequences of Slit proteins are conserved during evolution, the functional roles of the C-terminal part within Slit or the cleaved C-terminal fragment remain unclear. Removal of the C-terminal part from Slit does not eliminate Slit binding to its receptor, or its signaling in axon guidance and neuronal migration [23•,24•,26•]. There is indirect evidence that the C-terminal part may regulate Slit diffusion [23•]. Heparan sulfate proteoglycans are important for high-affinity binding of Slit to its receptor and for the repulsive activity of Slit [27•]. Biochemically, Slit-C has higher affinity than Slit-N in binding to the heparan sulfate proteoglycan, glypican-1 [28,29]. Whereas expression of glypican-1 in the mammalian spinal cord is similar to that of Slit-2 [29], the function of glypican-1 in either axon guidance or cell migration has not been established.

Roundabout: a receptor for slit

Roundabout (Robo) was discovered in a genetic screen for mutations affecting axon pathfinding in *Drosophila* [30]. *robo* mutants exhibit an increased number of axons crossing and re-crossing the ventral midline [30,31]. cDNAs for *Drosophila* and *C. elegans* Robos were isolated in 1998 and showed that Robo encodes a single-pass transmembrane receptor [31,32]. Its extracellular region contains five immunoglobulin (Ig) domains and three fibronectin type III repeats. The large intracellular region of *Drosophila* Robo contains four identifiable conserved motifs designated CC0, CC1, CC2 and CC3 [31,32] (Figure 1). Three Robo genes have been identified in organisms including *Drosophila* [31,33•,34••,35•,36••], *C. elegans* [32], the zebrafish [37,38], the mouse [4,13,39] and the human [31]. It should be noted that, although Robos from other species are similar to the prototypical *Drosophila* Robo, *Drosophila* Robo-2 and -3 lack the CC2 and CC3 motifs [33•,34••,35•,36••].

Robo can be proteolytically cleaved in cultured cell lines [6••,14], but the cleavage site has not been determined and its *in vivo* significance is unknown. In the extracellular part, the Ig domains in Robo are sufficient for binding to the full-length and the LRRs of Slit [23•,24•,26•]. The intracellular part of Robo determines the repulsive response to Slit [40]. Deletion of each of the CC motifs compromises, but does not eliminate, the function of Robo [41••], suggesting that these motifs play significant but redundant roles.

Function of Slit and Robo in axon guidance and dendrite arborization

Slit–Robo signaling regulates axon pathfinding at the ventral midline of the neural tube and controls the projection of axons in multiple additional regions in vertebrates. How the slit signal controls axon guidance has been revealed in an avalanche of recent publications from a broad spectrum of experimental systems (Figure 2).

In the anterior neural tube, axons from the olfactory bulb project ipsilaterally in the lateral olfactory tract and do not cross the septum, a midline structure in the forebrain. A repulsive activity in the septum affects these axons [42]. Slit-1 and -2 are both expressed in the septum [6] and are candidates for the repulsive signal [6,10]. Although application of the extracellular part of Robo failed to block the repulsive activity in the septum [43•,44•], the repulsive activity in the septum is removed in mice lacking both Slit-1 and -2 genes and the olfactory bulb axons abnormally cross the midline in these knockout mice [45••,46••] (see 'Note added in proof'), indicating that endogenous Slit-1 and -2 proteins are essential for midline guidance in the forebrain. In the neocortical midline, Slit constitutes a repulsive signal emanating from the glial wedge for neocortical axons which is important for the formation of the corpus callosum [47•]. In Slit-2-deficient mice, most callosal

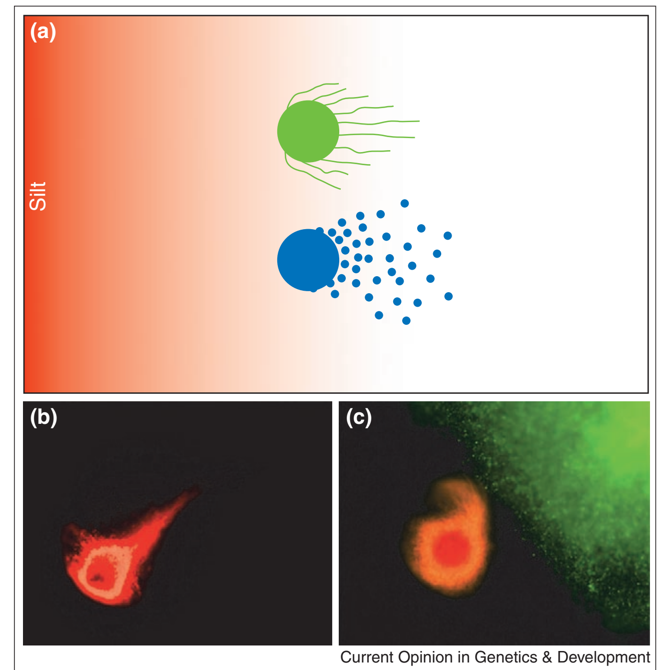
axons fail to cross the midline through the corpus callosum [45••]. The deficient mice reveal a redundant role for Slit-1 and Slit-2 in guiding the midline crossing and in positioning the dorsoventral aspect of major axonal pathways such as the corticothalamic and thalamocortical projections [45••].

In the spinal cord, commissural axons originate in the dorsal part are attracted to the ventral midline (the floor plate) where their responsiveness to the attractant is silenced. The commissural axons cross the floor plate and then turn longitudinally on the contralateral side. Netrin is the attractant synthesized in the floor plate [48–50], whereas Slit may play three roles in guiding commissural axons. First, Slit may silence the response of commissural axons to Netrin once the axons reach the floor plate by inducing the interaction of Robo with DCC (Deleted in Colorectal Cancer), a receptor known to mediate the attractive response of Netrin [51••]. Second, Slit is a repellent for preventing commissural axons that have crossed the floor plate from re-crossing. Genetic data in *Drosophila* and *C. elegans* indicate that Slit and Robo are required for preventing re-crossing [5,9,15•,30,31]. In mice, all three Slit genes are expressed in the floor plate [6,13] and gene-targeting of Slit-1 and -2 did not affect commissural crossing [45••,46••]. Perhaps all three Slit genes have to be knocked-out before a role for Slit in the spinal cord can be determined. The third role for Slit in commissural axon guidance is in longitudinal turning. Based on the expression of Slit2 in both the floor plate and the motor neuron columns [6], it was proposed that commissural axons that have crossed onto the contralateral side may turn longitudinally between the floor plate and the motor neurons [6]. Work on Slit2 and semaphorins demonstrate that both types of molecules may contribute to longitudinal turning [52••]. Different roles of Slit in guiding commissural axons depend on changes in the responsiveness of commissural axons to Slit. The expression of Robo on the cell surface is tightly regulated [31,53]: Robo expression is reduced significantly when commissural growth cones extend into the midline [31,53], whereas Robo is dramatically increased on the cell surface once the axons have crossed the midline [31,53]. Robo expression pattern can explain the multiple function of Slit signaling in commissural guidance.

Slit and Robo are also involved in positioning the longitudinal tracts. In the *Drosophila* CNS, axons are organized into three parallel longitudinal tracts on each side of the midline. Expression of different Robo receptors by these axons specifies their relative positions [33•,34••,35•,36••]. The medial axons express Robo, the intermediate axons express Robo and Robo3, whereas the lateral axons express Robo, Robo2 and Robo3. Slit appears to function as a short-range signal that prevents axon re-crossing at the midline and as a long-rang cue that patterns the longitudinal pathways by signaling through a combinatorial code of Robo receptors [33•,34••,35•,36••].

Spatial and temporal patterns of Slit and Robo expression and functional data suggest roles for Slit–Robo signaling in

Figure 2



Functions of Slit (red gradient) in the CNS. **(a)** Slit can function as a chemorepellent on navigating axons (green explant) and on migrating neurons (blue explant). Axons or neuronal precursor cells move down a Slit gradient, away from the source of Slit, indicating the repulsive activity of Slit on these cells. **(b,c)** Fluorescent images of DiI-labelled (red) lateral olfactory tract (LOT) in E15 rat brains. (c) When overlaid with a cell aggregate expressing Slit (labeled green by DiO), the projecting axons turned away from the Slit source, indicating Slit can repel axons from the LOT in their natural environment. ([b] The same type of culture overlaid with control cells.)

the developing visual system. Slit can repel and inhibit the outgrowth, induce growth cone collapse and increase the fasciculation of RGC axons [54•–56•]. Mutations in zebrafish Robo2 cause defects in retinal–tectal projection, excessive midline crossing and defasciculation of the RGC bundles [57•,58••]. Robo2 is important in preventing and correcting pathfinding errors in zebrafish RGC axons [57•,58••]. In mice lacking both *slit-1* and *-2* genes, the optic chiasm is located ectopically [46••]. There were also ectopically projecting fibers throughout the retina and the optic chiasm [46••]. *In vitro* evidence indicates that Slit can also repel motor axons [4], hippocampal axons [10] and cortical axons [10,59••].

Slit has been biochemically identified as a positive regulator of branching and elongation of axons from the DRG [7]. Recent findings showed that Slit can also induce branching and arborization in central trigeminal axons in the brainstem [60]. Exposure of central trigeminal axons to Slit2 during the elongation phase causes pre-mature branching and arborization of these axons and this effect can be blocked in the presence of the Robo ectodomain [60]. Together, these findings suggest an important role of Slit in branching and arborization of CNS sensory axons.

Data from *in vitro* experiments provided evidence for the positive effect of Slit-1 on the growth and branching of dendrites from cortical neurons [59••]. Addition of Robo1 or Robo2 ectodomain in slice culture overlay assays significantly decreased the number of dendritic branches in both nonpyramidal and pyramidal cells [59••]. These data suggest that endogenous Slit can promote dendritic growth and branching. Slit-1 is initially expressed in the cortical plate at E15 [59••]. Whitford *et al.* have hypothesized that the role of Slit-1 on cortical neurons at this stage is axon repulsion. The effects of Slit-1 on axon repulsion and dendrite branching were seen in E18 cortical neurons [59••]. The mechanism by which different poles of cortical neurons (i.e. axons and dendrites) respond differentially to the same molecule, Slit-1, remains an interesting question to be addressed.

Slit guidance of neuronal migration

Neuronal migration is important in both the developing and the adult CNS. Neuronal precursor cells migrating in the radial direction rely on glial fibres whereas those migrating in the tangential direction do not. The glial fibres do not provide directional information to the migrating neurons. Studies of Slit *in vitro* demonstrate that the same guidance cue regulating axon pathfinding could control tangential neuronal migration [8]. Further studies confirmed this [11,12] and extended the role of Slit to radial migration [11].

In the developing mammalian forebrain, neuronal precursor cells from the anterior subventricular zone (SVZa) migrate into the olfactory bulb to form interneurons [61]. A repulsive activity for the SVZa cells was found in the septum [62]. Slits are expressed in the septum during these developmental stages [8] and can repel SVZa cells both in collagen matrices and in brain slices [8,11]. Application of a truncated Robo (RoboN) containing only the extracellular domain of Robo, reduced the repulsive activity in the septum [8].

Slit also repels cells migrating from the ganglionic eminences (GEs) to the neorcortex [12]. Neurons in the neocortex were traditionally thought to originate there until recent studies revealed that GABAergic neurons, the major type of cortical interneurons, have an extracortical origin in the subventricular zone of the GEs [63]. The ventricular zone of the GEs is repulsive to the GABAergic interneurons [12] and Slits are expressed in the ventricular zone of the GE [12]. Slit is repulsive to the GABAergic neurons and RoboN could reduce the repulsive activity in the ventricular zone [12]. In addition to guiding the tangential migration of the SVZa an the GE cells, *in vitro* studies show that Slit can also repel the ventricular zone cells from the neocortex [11], which is normally dependent on glial fibres *in vivo*.

We have proposed that Slit functions as a repellent by forming a concentration gradient and that cells migrate down the Slit gradient [8]. Those suggestions came from work applying Slit from a point source placed on one side of the migrating cells [8,12]. Mason *et al.* [64•] have recently applied Slit in a uniform concentration and found that Slit could inhibit cell migration. They suggested that Slit could only function as an inhibitor but not as a repellent [64•]. They found a glia-derived soluble factor, migration-inducing activity, which can promote SVZa cell migration [64•], and proposed that only the combination of migration-inducing activity and Slit can repel the SVZa cells. Although there is indirect evidence to support the role of Slit itself as a repellent [8,12], further investigations are necessary to establish directly whether Slit is a repellent.

It should be noted that, although Slit can repel SVZa cells, GABAergic neurons, and ventricular zone precursor cells *in vitro*, there is so far no evidence to prove that endogenous Slit guides the migration of any mammalian cells *in vivo*. Gene targeting of Slit-1 and -2 did not show a phenotype in SVZa migration [45••,46••]. There could be redundancy among all three Slit proteins, or redundancy of Slit with other guidance molecules in controlling the migration of neuronal precursor cells in mammals. In *C. elegans*, there is only one *slit* gene [15•] and genetic evidence support an *in vivo* role for *slit* in neuronal migration. CAN and ALM neurons in the head of *C. elegans* embryos migrate posteriorly. *Slt-1* mutants show defects in the posterior migration of these neurons, resulting in their premature stop in the middle of their migratory tracks [15•].

Control of non-neuronal cell migration by slit

Many cell types migrate in developing or adult animals. In mammals, expression of both *slit-2* and *-3* genes outside the nervous systems in adult animals has been detected [21••]. *robo* genes are also expressed in non-neuronal cells including leukocytes [21••]. Early work on *Drosophila* implicated Slit in muscle positioning [2,3]. Recent work has shown that Slit may function in at least two stages of muscle precursor cell migration [65•]. During *Drosophila* myogenesis, muscle precursors migrate away from the midline to the periphery. These precursor cells then fuse to form muscle fibres and extend growth-cone-like structures toward target muscle attachment sites within the epidermis. In *slit* and *robo* mutants, muscle precursor cells fail to move away from the midline and some of them fuse across the midline [3,65•]. When this early defect is rescued — by expressing Slit protein specifically in the midline cells in *slit* mutants — the precursor cells can migrate to the periphery, but they end up being attached to wrong muscle attachment sites in the epidermis, indicating that the normal expression of Slit in the attachment sites are attractive for the myofibers [65•]. These results indicate that Slit may function as a repellent during the early phase of muscle precursor cell migration and as an attractant during the late phase of *Drosophila* myofiber attachment to the epidermis. It remains unknown how the muscle response to Slit switches from attraction to repulsion.

Whereas cells such as muscle precursor cells in the embryo are not dramatically different from neuronal precursor

cells, chemotactic leukocytes are very different from migrating neurons. Neurons migrate in pathways (and environments) different from those by leukocytes; neuronal migration is much slower than leukocyte chemotaxis; migrating neuronal precursor cells have specialized, relatively long and single leading and trailing processes whereas leukocytes have short and multiple pseudopodia; neurons have both positive and negative guidance cues whereas only positive chemotactic factors were known for leukocytes; all known neuronal guidance cues use single transmembrane receptors whereas all chemotactic factors function through seven-pass transmembrane receptors coupled to G proteins. It therefore seems that distinct mechanisms could have evolved to guide the migration of different types of cells. Recent work on Slit, however, has provided strong evidence that guidance mechanisms are conserved in cell types as different as neurons and leukocytes [21••].

In a typical leukocyte chemotaxis assay, leukocytes are placed in the upper chamber that is separated from the lower chamber by a filter with defined pores. When a chemoattractant such as the chemokine stromal-derived factor-1 (SDF-1) is added to the lower chamber, the number of cells migrating to the lower chamber is increased. Slit can block the attractive effect of SDF-1. When SDF-1 is placed in the lower chamber while Slit is placed in the upper or the lower chamber, the attractive effect of SDF-1 is eliminated, indicating that Slit can inhibit leukocyte chemotaxis induced by chemokines. Because Slit is effective even when it is present in both the upper and the lower chambers, the inhibitory effect of Slit does not seem to require a concentration gradient [21••]. Slit has similar inhibitory effects on multiple types of leukocytes including lymphocytes, monocytes and neutrophils. The inhibitory signal is likely to be mediated by Robo because it was blocked by the ectodomain of Robo and the antagonism between Slit and SDF-1 can be reconstituted in human embryonic kidney (HEK) cells expressing both Robo and CXCR4, the receptor for SDF-1 [21••]. The presence of Slit and Robo in multiple cell types from epithelial cells in the kidney to endothelial cells in the vasculature support a general role for Slit–Robo signaling in controlling the positioning and migration of a variety of mammalian cells.

Finally, Slit–Robo signaling may direct the movement of epithelial sheaths. Genetic studies in *Drosophila* demonstrated that *leak*, a robo2 receptor, is important for proper head development [66]. *leak* mutants show defects in the formation of the larval cuticle. The expression pattern for *leak* mRNA suggests that it functions as a receptor for slit during the development of the *Drosophila* head and that slit may function as an attractive cue in guiding dorsal closure of the ectoderm [66].

Intracellular transduction mechanisms for Slit–Robo signaling

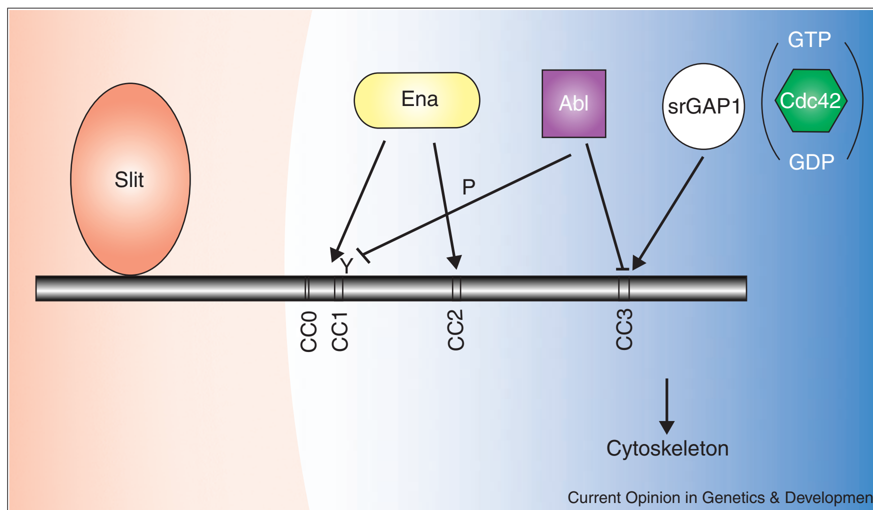
We are just beginning to discover intracellular components involved in the Slit–Robo pathway. Although some components may participate in Slit–Robo signaling directly and others may modulate the pathway, it is not easy to distinguish between these activities.

Work in *Drosophila* indicates that the Abelson (Abl) kinase and the Enabled (Ena) protein are involved in Slit–Robo signaling [41••]. *abl* and *ena* mutants show genetic interaction with *slit* and *robo* [41••]. The CC1 and CC2 motifs of Robo are important for its biochemical interaction with Ena whereas the CC3 motif is important for its interaction with Abl. Abl is a negative regulator of robo: overexpression of Abl caused midline guidance phenotypes that resembled *robo*$^{-/-}$ in a dosage-sensitive and kinase-dependent manner [41••], suggesting an inhibitory effect of Abl on Robo. Tyrosine phosphorylation of Robo by Abl is important for the inhibitory effect of Abl on robo [41••]. The phenotypic interactions between *ena* and *robo* indicate that they function in similar directions, rather than antagonistically [41••]. It is not clear how Slit–Robo signaling can cause repulsion through Ena, although Ena is thought to promote the formation of long actin fibres in a geometry that does not favor cell migration [67,68]. Loss of either *abl* and *ena* function does not phenocopy *slit* or *robo* mutants, indicating that neither of them is dedicated to the Slit–Robo pathway [41••]. It remains to be determined whether Abl and Ena are signal transducing components or modulators of the Slit–Robo pathway.

Recently, a family of GTPase-activating proteins (GAPs) named srGAPs have been identified [69••]. *srGAP-1* and *-2* mRNA are expressed in regions responsive to Slit, in a pattern similar to that of Robo-1 in the mammalian CNS. Each srGAP contains an FCH domain, an SH3 domain and a GAP domain for the Rho family of small GTPases, which include Rho, Rac and Cdc42. The function of the FCH domain in Slit–Robo signaling is unknown. The GAP domain is responsible for inactivating Cdc42 and RhoA in HEK cells [69••]. In HEK cells, srGAP1 can bind to and decrease the level of active Cdc42 and RhoA, but not Rac1. SH3 domains are involved in binding to the CC3 motif in Robo. When an srGAP1 mutant lacking the GAP domain was introduced into SVZa neurons, the repulsive response to Slit was blocked [69••], indicating a role for srGAP1 in Slit–Robo signaling.

Extracellular application of Slit can increase the intracellular interaction between srGAP1 and Robo. Slit can increase the interaction between srGAP1 and Cdc42, but decrease its interaction with RhoA. The regulation of RhoA and Rac1 by Slit is cell-type-dependent, and their roles in Slit signaling have not yet been established. Slit treatment consistently inactivates Cdc42 in both the HEK cells and the SVZa neurons [69••]. To test the hypothesis that cdc42 is a target of slit signals, a constitutively active Cdc42 was introduced into SVZa cells. The repulsive effect of Slit on migrating SVZa cells was lost in neurons expressing the constitutively active CDC42, indicating that inactivation of Cdc42 is essential for mediating the repulsive Slit–Robo

Figure 3



Intracellular signal transduction pathway for Slit and Robo. The protein Ena interacts with Robo through the CC1 and CC2 regions and its function may contribute to Robo repulsion. The non-receptor tyrosine kinase, Abl, interacts with Robo perhaps through the CC3 region. Abl may function as an antagonist of Robo by phosphorylating Robo at a tyrosine residue located in CC1. Data from other biochemical studies show that Ena can also be a substrate of Abl. srGAP1 regulates the activities of the RhoGTPases, including Cdc42. Slit enhances the association between srGAP1 and Robo via the CC3 motif and this localization may induce a local inactivation of Cdc42. Together, these molecules may either directly or indirectly introduce changes in the cytoskeletal components, including the re-organization of the actin and microtubule networks, and lead to directed cell migration.

signaling during neuronal migration [69••]. On the basis of Cdc42 work, we have proposed a signal transduction pathway that leads from Slit–Robo interaction to actin polymerization (Figure 3).

Genetic studies in *Drosophila* further suggest that the receptor tyrosine phosphatases (PTPases) PTP10D and PTP69D may modulate the Slit–Robo pathway [70]. *Drosophila* mutants lacking *robo*, *ptp10D* and *ptp69D* exhibit a severe phenotype that resembles the *slit* [70]. In *ptp10D ptp69D* mutant animals lacking one copy of *slit*, the midline repulsion defect is enhanced [70]. These results indicate that PTP10D and PTP69D positively regulate the Slit–Robo pathway, possibly to reverse the affects of the Abl kinase.

Recent discoveries reported by Stevens and Jacobs [71••] showed the genetic interactions between the integrin and Slit signaling pathways in *Drosophila* midline guidance. *Drosophila* embryos doubly heterozygous for *slit* and one of the integrin genes or an integrin ligand resulted in ectopic longitudinal axon trajectories. These data suggest that integrins may positively regulate the responsiveness of these growth cones to Slit.

The metalloprotease, Kuzbanian, has also been implicated in Slit–Robo function during growth cone navigation in *Drosophila* midline [66]. Genetic evidence showed that *kuzbanian* genetically interacts with *slit*. Immunohistochemistry data demonstrated that the expression of a dominant negative Kuzbanian protein in midline cells resulted in a failure in the clearance of the Robo receptor from commissural axons [66]. These results suggest a role for the proteolytic activity of Kuzbanian in activating the Slit–Robo receptor complex.

Cyclic nucleotides can regulate responses to all known attractive and repulsive guidance cues [72]. The Slit response seems to be regulated by cyclic GMP [73•]. When the DRG explants were presented with alternating stripes of Slit2-N and fibronectin, DRG axons preferentially grew into stripes coated with Slit2-N [73]. In the present of a protein kinase G inhibitor, the response of DRG axons to Slit2-N/fibronectin was reversed. It remains unknown which step(s) is regulated by the cyclic nucleotides.

Conclusions

One of the most interesting suggestions from studies of Slit is that there is a conserved mechanism for guiding the migration of cells as distinct as neurons and leukocytes. This illustrates that intensive studies of some molecules can lead to findings of mechanistic implications in a context broader than that of a single molecule. It should be noted that the spectrum of Slit function and the responsive cell types are still not clear. Although most roles for Slit–Robo are related to motility, there is a report of Slit function in asymmetric cell division [74•] that cannot be easily integrated into a simple picture of how Slit–Robo functions. The *in vivo* roles of Slit–Robo in mammals are only partially known, perhaps owing to redundancies among multiple *slit* and *robo* genes.

The multiple roles of Slit in axon guidance is exemplified in the context of commissural axons in the spinal cord, where the roles of Slit changes as the axons elongate further. It should be noted that it is still unclear what regulates Robo expression and the response of commissural axons to Slit.

Although a hypothetical signal transduction pathway has been proposed for mediating Slit–Robo signaling, it is clear that we do not know all the components in the pathway and how they are modulated by other molecules. Studies of repellents may provide an exciting alternative to attractants

for investigations of mechanisms underlying polarized cell migration.

When the roles of Slit and mechanisms of Slit–Robo signaling are studied in broader contexts such as neural development and leukocyte trafficking, we may also gain insights into physiological processes such as development and pathological processes such as inflammation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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of mutant growth cones demonstrated that Robo2 signaling occurs before and after midline crossing. Time-lapse microscopy of these mutant axons revealed an additional role of Robo2 during pathfinding: error correction of stray axons. In wild-type animals, retinal axons make pathfinding errors that are subsequently corrected. In *astray* mutants, these errors occur more frequently and they persist and are corrected at a much lower frequency.

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The authors reported an inhibitory effect of Slit on SVZa cells in the presence of a uniform Slit concentration. It was concluded that Slit acted purely as an inhibitor instead of a repellent for migrating SVZa cells. A glia-derived factor was purified and shown to induce SVZa migration. Only a combination of this glial factor and Slit was proposed to function as a repellent.

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The authors reported the identification of a novel family of GAPs in the Slit–Robo signaling pathway. Through these GAPs, Slit may downregulate RhoGTPases such as Cdc42. It remains to be investigated how this eventually leads to neuronal repulsion.

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Genetic studies in *Drosophila* suggest that Slit promotes the asymmetric division of neural precursor cells by downregulating certain proteins. In *slit* mutant, cell division of these neural precursors became symmetrical. The protein levels of Nubbin and Mitimere, whose downregulation allows the asymmetric localization of Inscuteable and leads to asymmetric division, were not changed nor downregulated before cell division in slit mutants. These data indicated that slit may play a role in promoting terminal asymmetric division in these neural precursors by the regulation of target protein levels and localization.

Note added in proof

75. Nguyen-Ba-Charvet KT, Plump AS, Tessier-Lavigne M, Chédotal A:
• **Slit1 and Slit2 proteins control the development of the lateral olfactory tract.** *J Neurosci* 2002, **22**:5473-5480.

This paper shows that deletion of both *Slit1* and *Slit2* genes results in abnormal projection of the olfactory bulb axons, supporting the suggestion made in [6,10] on the role of Slits in olfactory bulb axon guidance. These results also indicate that caution should be made of results from using soluble Robo ectodomains [43•,44•]. One possibility is that soluble Robo ectodomains are limited in diffusion, and another possibility is that they were applied too late.