The cell biology of neuronal navigation

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Morphogenesis of the nervous system requires the directed migration of postmitotic neurons to designated locations in the nervous system and the guidance of axon growth cones to their synaptic targets. Evidence suggests that both forms of navigation depend on common guidance molecules, surface receptors and signal transduction pathways that link receptor activation to cytoskeletal reorganization. Future challenges remain not only in identifying all the components of the signalling pathways, but also in understanding how these pathways achieve signal amplification and adaptation—two essential cellular processes for neuronal navigation.

Neuronal navigation during early development is essential for establishing the highly ordered cellular organization and specific nerve connections in the nervous system[1,2]. In the developing nervous system, newly generated cortical neurons in the ventricular zone migrate along the surface of radial glia fibres and settle in the cortical plate to form orderly layers of the cortex[3,4]. Neurons generated in subcortical structures also undergo long-range tangential migration to the cortex to form dispersed population of cortical interneurons[4]. Neural crest cells that emerge from the dorsal margin of the neural tube migrate over long distances along specific routes to form sensory, autonomic and enteric ganglia in the peripheral nervous system[5]. After reaching its destination, each neuron develops a set of dendrites characteristic of its phenotype and a single long axon that extends along specific routes to reach prospective synaptic partners[6].

The migration of either the entire neuron or its nerve growth cones is guided by the interaction between the neuron and its local environment. Both forms of navigation use similar guidance molecules and cytoplasmic mechanisms for detecting and transducing guidance signals. Furthermore, many of the signal transduction events underlying neuronal navigation are remarkably similar to those responsible for the chemotaxis of both leukocytes and *Dictyostelium discoideum* amoebae[7]. Aided by the comparison with findings on the mechanisms of chemotaxis in non-neuronal cells, we review general cell-biological issues of directed cell motility that are relevant to both neuronal migration and axon guidance. For more details on various aspects of neuronal navigation, several recent, comprehensive reviews may be consulted[4,8,9].

Historical overview

The developing tissue may influence neuronal navigation by non-specific physical constraints and specific chemical signals. Soon after his invention of the tissue-culture technique, Harrison[10] observed that cultured amphibian neuroblasts prefer to cling to and move along solid surfaces. Weiss[11] later showed that migrating embryonic cells and growing neurites from cultured explants tend to move along scratches on the glass culture plate, or along lines of tension in the plasma clot embedding the explant. He proposed that mechanical (contact) guidance, or 'stereotropism', is the main factor in directing neuronal motility. The idea of contact guidance was later refined by incorporating differential adhesiveness as a factor in guiding migrating cells[12]. Sperry[13] further argued that highly specific nerve connections can only be achieved by specific chemical affinities between growing axons and their target cells, and precise topography of connections can be established by matching gradients of molecular cues carried by axons and their targets.

More recent studies have brought new insights into two aspects of contact guidance: the molecular basis of differential adhesion and the role of cytoplasmic signalling. Several families of cell-surface adhesion molecules have been shown to be important in neuronal navigation[14]. For example, axonal growth along 'pioneering' axons, 'guidepost' cells, or 'labelled' pathways in the developing embryos[15,16] can be attributed to selective adhesion owing to the presence of specific cell-adhesion molecules (CAMs) on the neuronal surface[14]. The migration of postmitotic cortical neurons along radial glia depends on the presence of specific neuronal surface proteins, such as astrotactin[17] and integrins[18,19]. Rostral migrating neurons from the subventricular zone can also adhere to one another through the neural adhesion molecule (N-CAM) to form the migratory stream to olfactory bulb[20].

As most adhesion molecules are transmembrane proteins, cell–substrate interaction might involve not only the formation of adhesive bonds, but also the activation of cytoplasmic signalling cascades, leading to 'trophic' or 'modulatory' actions on neuronal functions. Indeed, N-CAM and L1 are known to trigger cytoplasmic signalling that activates various kinases and elevates levels of Ca^{2+}—processes that regulate neuronal motility[14]. Binding of extracellular matrix (ECM) molecules (for example, laminin and fibronectin) to neuronal surface integrins is required for proper neural crest migration[21]. Laminin also supports neurite outgrowth from cultured explants[22] and modulates the navigation behaviour of growth cones in response to guidance cues[23]. The modulatory action of cell–substrate contacts can be negative as well: the migration of trunk neural crest cells is restricted to a path on the rostral half of each somite, apparently owing to repulsive action of ephrins expressed by the caudal halves of the somites[24].

Thus, it is now well established that two contact-mediated processes — selective adhesion and intracellular signalling—have critical roles in determining the neuron's response to its environment. Note that any contact-mediated event involving neuron–substrate interaction begins with selective 'adhesion' (the initial formation of molecular bonds); however, the outcome of the interaction, which can range from stable adhesion to repulsion of the neuron from the substrate, depends on subsequent cytoplasmic signalling. This is nicely illustrated by the interaction between surface-bound ephrins and their Eph receptors, which begins with selective adhesion but ends with repulsion, a sequence of events requiring the cytoplasmic signalling of activated Eph receptor and the release of metalloprotease that cleaves the ephrin from the surface[25].

Intrigued by the amoeboid morphology of the nerve growth cone and the phenomenon of leukocyte chemotaxis, Ramon y Cajal[26] suggested more than a century ago that the migration of neuroblasts and growth cones might be guided by gradients of chemical substances secreted by the target cells. This idea of 'chemotropism' received little attention until co-culture experiments[27–30] and *in vivo* observations[30–32] provided strong evidence for the existence of target-derived factors that can act at a distance to attract or repel axons. Several families of secreted proteins,

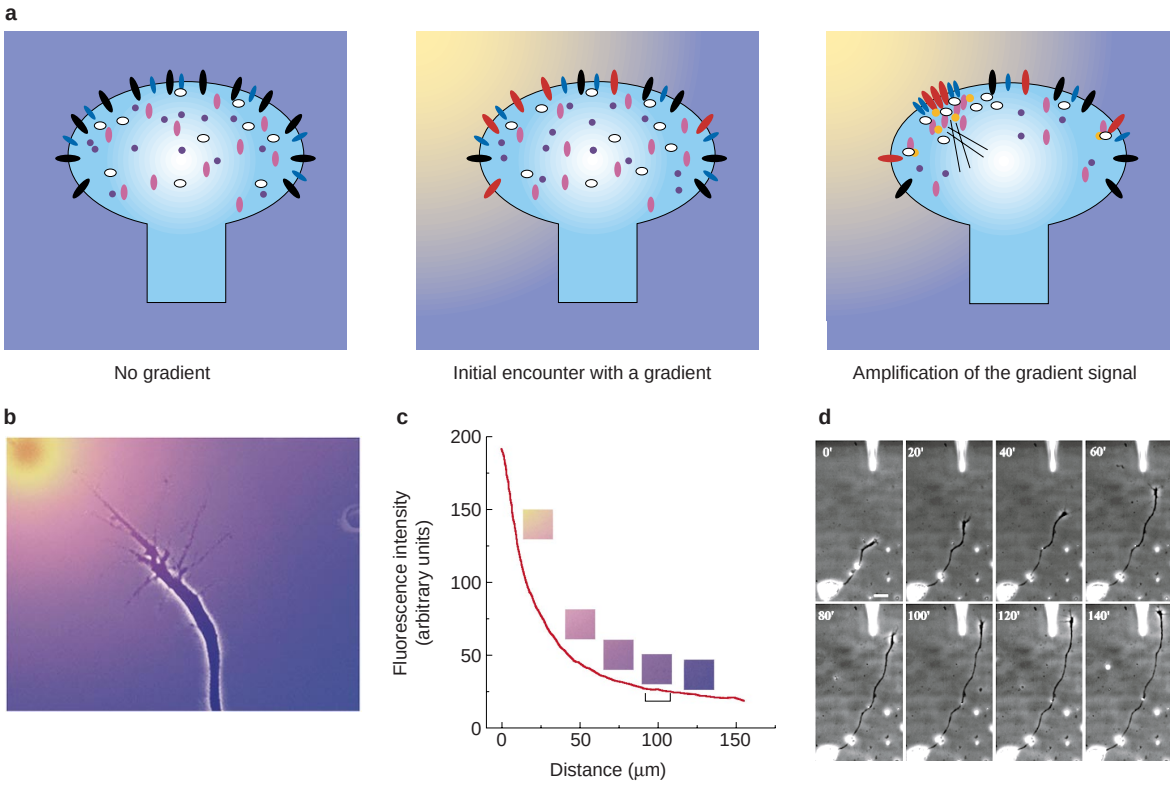

Figure 1 **Amplification and adaptation during growth cone navigation in a gradient of guidance signals. a**, A model for signal amplification at the growth cone, based on a cascade of clustering signalling molecules. In encountering a shallow gradient of guidance molecules (yellow cloud), a shallow gradient of receptor activation (red oval) leads to an autocatalytic clustering of activated receptors and/or their downstream effectors in the membrane. The initial clustering triggers the recruitment and further clustering of cytoplasmic adaptors and effectors, leading eventually to orientated assembly of actin filaments on one side of the growth cone. The cascade of local clustering and consequent depletion of the signalling molecules at the rest of the cell provide the local activation and global inhibition required for the amplification of the gradient signal. **b**, Chemotropic turning of the growth cone of a cultured *Xenopus* spinal neuron in a gradient of diffusible guidance molecule netrin-1. The gradient was produced by repetitive pulsatile application of picolitres of solution containing netrin-1 and visualized by co-ejection of a fluorescent marker. **c**, Curve depicting the concentration of the guidance molecule with distance from the pipette tip, as determined by the intensity of the marker fluorescence. Chemotropic extension of the growth cone in such a gradient requires the growth cone to continuously re-adjust its sensitivity to the gradient as the basal level of the guidance molecule increases by several orders of magnitude. Insets associated with the curve represent gradients encountered by the growth cone (10 μm in width) at different distances from the source of netrin-1. **d**, Adaptation of the growth cone response in a netrin-1 gradient, as shown by the 'zigzag' pattern of the growth trajectory of a typical fast growing neurite of *Xenopus* spinal neuron in culture. (Data adapted from ref. 71.). The alternating attractive and repulsive turning of the growth cone indicates cycles of desensitization and resensitization to the growth cone towards netrin-1. Scale bar, 20 μm.

including netrins, semaphorins, slits, neurotrophins and fibroblast growth factors, have now been identified to be diffusible guidance molecules for neuronal navigation in various regions of the brain[8,9,33]. As discussed below, long-range chemoattraction and chemorepulsion may indeed occur in the nervous system, but whether these secreted proteins act as diffusible factors or as factors bound to ECM and cell surfaces remains unclear.

The nature of guidance signals

A guidance factor for neuronal navigation generally fulfills the following criteria. First, it is expressed or secreted at the right time in the appropriate regions of the nervous system to serve as an attractive/repulsive function. Second, depletion of the factor or its receptor by genetic mutation or other means leads to navigational errors of the neuron in question. Third, expression or secretion of the factor by cultured cells is sufficient to induce attraction/repulsion of co-cultured neurons or growth cones. In further analysis of nature and cellular actions of the guidance signal, however, one must consider the following issues.

Directional versus positional signals. The information regarding which direction to grow and when to stop must be imparted to the neuron or axon, but it is not always clear whether a guidance molecule is providing one or both of these signals. A case in point is the role of ephrins and Eph receptors in guiding retinal axons to their tectal targets[32,34–36]. Gradients of ephrins found in the tectum can, in principle, provide both directional and positional information[37,38]. Experimental evidence indicates that complementary ephrin–Eph receptor gradients in the retina and tectum can serve as positional signals, but whether they also serve as directional signals remains to be determined[38]. Discrete stop signals are also found for neuronal migration. Radial migration of Purkinje neurons in developing cerebellum seems to be inhibited by the presence of reelin[39], a putative positional guidance cue secreted by cells in the marginal zone. Whether reelin also provides a directional signal for the migration is not clear.

Many factors are required for neuronal navigation, but provide neither directional nor positional information. Surface CAMs, in particular those in the immunoglobulin-γ superfamily, are essential for axon fasciculation, which in turn is required for proper axon pathfinding[40,41]. Mutation of cytoplasmic signalling molecules, such as cdk5 and p35, or transcription factors can also result in gross

alterations in neuronal navigation[39,42]. Studies of the function of permissive/modulatory factors, while helpful in revealing intricate cytoplasmic regulatory mechanisms, do not address directly the mechanism of transduction of guidance signals.

Many guidance molecules have been found to function as an attractant for one navigational event and a repellent for another[8,9,43]. Recent findings indicate that this bi-functionality can be attributed to differential receptor activation[44] or different levels of second messengers in the neuronal cytoplasm[43]. Interestingly, the same guidance cue can also serve for attractant and repellent for different parts (dendrite versus axon) of the same neuron[45]. Thus, the bi-functionality of guidance molecules reflects more the status of the neuron than an intrinsic property of the molecule.

Many identified guidance cues are secreted proteins and purified/recombinant proteins. Cell lines expressing the proteins have been used in co-culture experiments to show their effectiveness—as diffusible factors acting at a distance—in orienting the nerve growth or neuroblast migration. It is not clear in general, however, whether these secreted proteins are distributed in long-range gradients in the developing tissue, and whether they act as diffusible factors or as factors bound to ECM and cell surfaces. For example, because of their highly basic nature, secreted neurotrophins bind tightly to the surface of the secreting neuron[46] and produce localized synaptic modulation in culture[47]. Similarly, secreted Sema3A also seems to be concentrated mainly at the surface of the secreting muscle cell[48]. Although gradients of both diffusible or bound guidance cues can result in differential receptor occupancy across the neuronal surface, a diffusible ligand permits extensive lateral redistribution of ligand–receptor complexes, which in turn may lead cytoplasmic signalling events that are different from those triggered by the bound ligand.

An emerging theme in neuronal navigation is that axon guidance cues can also guide neuroblast migration. For example, netrin-1, a well-established diffusible axon guidance cue in many parts of the developing brain[9], also directs circumferential migration of basilar pontine neurons from their origin in the neuroepithelium to the ventral midline[49]. Slit, a midline chemorepellent for commissural axons[33], serves as a repellent for the migration of both interneuron precursors from the anterior subventricular zone in the telencephalon to the olfactory bulb, and GABA (γ-amino butyric acid)-containing neurons from an extracortical origin to the neocortex[50–52]. For membrane-bound guidance cues, ephrins serve as guidance signals for the projection of retinal axons in the optic tectum[38] and for the migration of trunk neural crest cells across the somite[24]. In all these cases, the same set of membrane receptors are involved in both axon guidance and neuroblast migration, suggesting that cellular transduction mechanisms for these two forms of neuronal motility may be similar. However, neuroblast migration involves active cytoskeletal reorganization not only at the advancing front (equivalent to the growth cone), but also at the rear end of cell. This may require long-range, global cytoplasmic signalling distinct from that associated with growth cone guidance.

Detection of positional and directional signals

In principle, target finding by migrating neuroblasts or growth cones can be achieved by random walks, which are guided only by constraints imposed by permissive substrates. The navigational task is completed when the neuron recognizes the stop (positional) signal provided by the target. There are examples of neuronal navigation that appear to depend only on the detection of a positional signal that instructs the neuron to grow, retract or stop. Postmitotic cortical neurons may simply migrate along the radial glia until they detect a stop signal to settle at appropriate positions and form the cortical layers. Neural crest cell migration may be channelled by permissive and repulsive ECM substrates until they recognize specific target regions in the embryo[21,24].

When neuronal navigation is guided by a long-range directional signal in the form of a concentration gradient of either diffusible or substrate-bound cues, however, the neuron must detect either (1) the gradient of the signal across the cell (or growth cone) by the difference in receptor occupancy on two sides of the cell surface (spatial detection), or (2) changes in the total level receptor activation with time as the neuron moves up and down the gradient (temporal detection). The slow speed of neuronal motility in general suggests that spatial detection is likely to be the main mechanism used in neuronal navigation.

An issue of interest is whether any signal that affects neuronal motility can serve as a directional signal when present in a gradient. Laminin, a ECM molecule that promotes adhesion and growth of neurites in culture, does not alter the direction of growth cone extension when applied to the neuron in a gradient[22]. A gradient of repellent tectal membrane (or ephrins) for the growth of retinal ganglion cell axons provides a 'stop' signal to the growing axon[53] without inducing clear repulsive turning of the growth cone when these cues exist in a smooth gradient[38]. Conversely, pharmacological inhibition of phosphatidylinositol-3-OH kinase (PI(3)K) abolishes the growth cone turning of *Xenopus* spinal neurons in a gradient of netrin-1, nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF), without affecting the overall rate of growth cone extension[54]. Thus, it seems that not all factors that increase or decrease neuronal motility can provide directional signal when present in a gradient. Conversely, factors that convey directional neuronal response may act on other effectors besides those that affect neuronal motility.

For long-range neuronal navigation based on the detection of gradients of attractants/repellents, the neuron is faced with two tasks: to respond reliably to small gradients across the cell (amplification), and to remain sensitive to the gradient when the average concentration of the guidance cues changes by many orders of magnitude as the neuron migrate up or down the gradient (adaptation)(Fig. 1). The minimal gradient of guidance cues required for the detection by the neuron sets the range by which the target cell can influence the navigating neuron by secreting attractants or repellents[55].For leukocyte chemotaxis, a gradient as low as 1% across the cell (10 μm in width) can be detected when the average concentration is near the dissociation constant (K_d) of the receptor–attractant complex[56].

Studies of cultured *Xenopus* spinal neurons[57–60] showed that the growth cone can respond to a gradient of diffusible attractants of about 5–10% across the growth cone, but the minimal required gradient was not determined. For substrate-bound gradients that inhibit growth cone extension, the growth cone of retinal ganglion neurons can detect about a 1% gradient of repellent tectal membrane fragments[61]. How does the neuron amplify such a small difference in receptor occupancy into a distinct directional cytoskeletal rearrangement? A common mechanism in gradient amplification in biological systems involves local self-activation and global inhibition[37,62]. Relatively persistent local elevation can be achieved by polarized translocation and clustering of cellular components, whereas depletion of these components in other regions of the neuron confers a simple form of global inhibition.

In migrating lymphocytes, chemokine receptors are localized to the leading front of the cell[63]. In neutrophils and amoebae, it was found that the receptor does not become polarized, instead there is a distinct polarized translocation of pleckstrin homology (PH)-domain-containing proteins[64–66], such as cytosolic regulator of adenylyl cyclase (CRAC) and Akt/protein kinase B (PKB), to some polarized binding sites on the inner face of plasma membrane. The binding and translocation of these cytosolic effectors to the membrane facing the source of the attractant seems to be the initial amplification of the guidance signal in these chemotaxing cells. Increased accumulation of phosphotyrosine has been found in the leading front of growth cone lamellipodia[67], but whether this reflects an initial event involved in signal amplification is not clear.

Table 1 Comparison of signalling components involved in axon guidance and chemotaxis of non-neuronal cells

	NGF-induced growth-cone turning	PDGF-induced cell migration	Chemokine-induced neutrophil chemotaxis	cAMP-induced amoebae chemotaxis
Essential	PI(3)K PLC-γ DAG Calcium PKC	PI(3)K PLC-γ DAG Calcium PKC Rac	PTX sensitive G proteins PI(3)K PLC	PTX sensitive G proteins PI(3)K Akt/PKB PKC PAK
Non essential	MAP kinase	MAP kinase Src		MAP kinase PLC
Potential modulators	cAMP	Ras-Gap Syp phosphatase		
References	54	99	100	7,66

PTX, pertussis toxin; PKC, protein kinase C; PLC, phospholipase C; DAG, diacylglycerol; PAK, p21-activated kinase, PDGF, platelet-derived growth factor.

Signal transduction: from receptors to cytoskeleton

Neuronal navigation is a highly specific process, requiring differential behaviours of different types of neurons. This specificity is conferred by the differential expression of specific membrane receptors for guidance molecules. Both guidance and permissive/modulatory signals received by the neuron are transduced by cytoplasmic signalling pathways and converge onto regulatory mechanisms for cytoskeletal rearrangement. Evidence described below suggests that the specificity in navigational behaviour resides mainly at the level of receptor and their ligand-dependent interactions, and that a set of 'adaptors' and 'mediators' are involved in linking activation of specific receptors to common cytoplasmic signalling pathways (Fig. 2). Many of these pathways appear to be similar to the directed migration of non-neuronal cell types (Table 1), even though the guidance cues and their receptors are distinctly different.

In chemotaxis of *D. dictyostelium* amoebae and other eukaryotic cells, such as neutrophils and macrophages, G-protein-linked proteins with seven transmembrane domains normally function as receptors for chemoattractants[7]. Receptors for neuronal navigation, by contrast, comprise a heterogeneous population of transmembrane proteins, including receptor tyrosine kinases, receptors with no recognized catalytic domains, and possibly receptor phosphatases[8,9,43]. The emerging picture from recent studies is that in most cases there are several receptors for each guidance cue and sometimes these receptors function as receptor complexes (Fig. 2).

Engagements of receptor complexes can control the specificity and the polarity of the response of the neuron to the guidance cue. For example, both deleted in colorectal cancer (DCC) and UNC-5 bind netrin-1, but interact with each other in the membrane[44]. Expression of UNC-5 in *Xenopus* spinal neurons converts DCC-dependent, netrin-1-induced attraction into repulsion, and this repulsion requires interaction of cytoplasmic domains of DCC and UNC-5, which is triggered by binding of netrin-1 to UNC-5 or DCC[44].

As semaphorin receptors, neuropilins and plexins both bind semaphorins with high affinity[68], and particular subtypes of both neuropilin and plexin are required for the response to specific semaphorins. In addition, L1 may be also a component for the receptor complex as L1 interacts with neuroplin-1 and is required for response to Sema3A[69]. For neuronal migration, many receptors have been found for reelin[70], although it is not clear whether these multiple receptors interact in the membrane and whether the interaction is relevant for regulating neuronal navigation.

Adaptors and Mediators.

Adaptors that interact specifically with the cytoplasmic domain of the receptors provide the link between diverse membrane receptors and common signalling pathways. These adaptors and their downstream effectors can serve as mediators of guidance signal or as permissive factors that modulate or gate signal transduction. Both yeast two-hybrid and genetic screening have identified many factors that interact with guidance cue receptors or affect guidance behaviours. To qualify as a mediator of directional guidance signals, a factor must satisfy at least the following criteria: (1) interference of its function disrupts neuronal navigation; (2) it is activated on reception of a guidance signal (by changing enzymatic activity or binding affinity); (3) exposure to the guidance signal results, at least transiently, in a gradientof its activity across the neuron or growth cone. For most of the putative mediators of directional signals shown in Fig. 2, the available evidence relates mostly to criteria (1) and (2). It is interesting to note, however, that many putative mediators for axon guidance are also implicated in chemotaxis of non-neuronal cells (Table 1). Examples of shared putative mediators are discussed below.

In cultured *Xenopus* spinal neurons, preventing an elevation in cytoplasmic Ca^{2+} levels abolishes the growth cone turning response in gradients of acetylcholine (ACh) or netrin-1[58,71,72]. Moreover, these gradients can induce a Ca^{2+} rise in the growth cone, and a transient Ca^{2+} gradient has been detected in some growth cones[58,72]. Photolytic release[73] of caged Ca^{2+} or induction of Ca^{2+} release from internal stores with an extracellular gradient of ryanodine[72] (in the absence of guidance cues) is sufficient to induce growth cone turning. Although a Ca^{2+} gradient has not been detected in migrating neurons, Ca^{2+} seems to be a mediator of directed neuronal migration as well[74].

In cell cultures, the turning responses of the growth cone induced by gradients of netrin-1, NGF, BDNF or myelin-associated

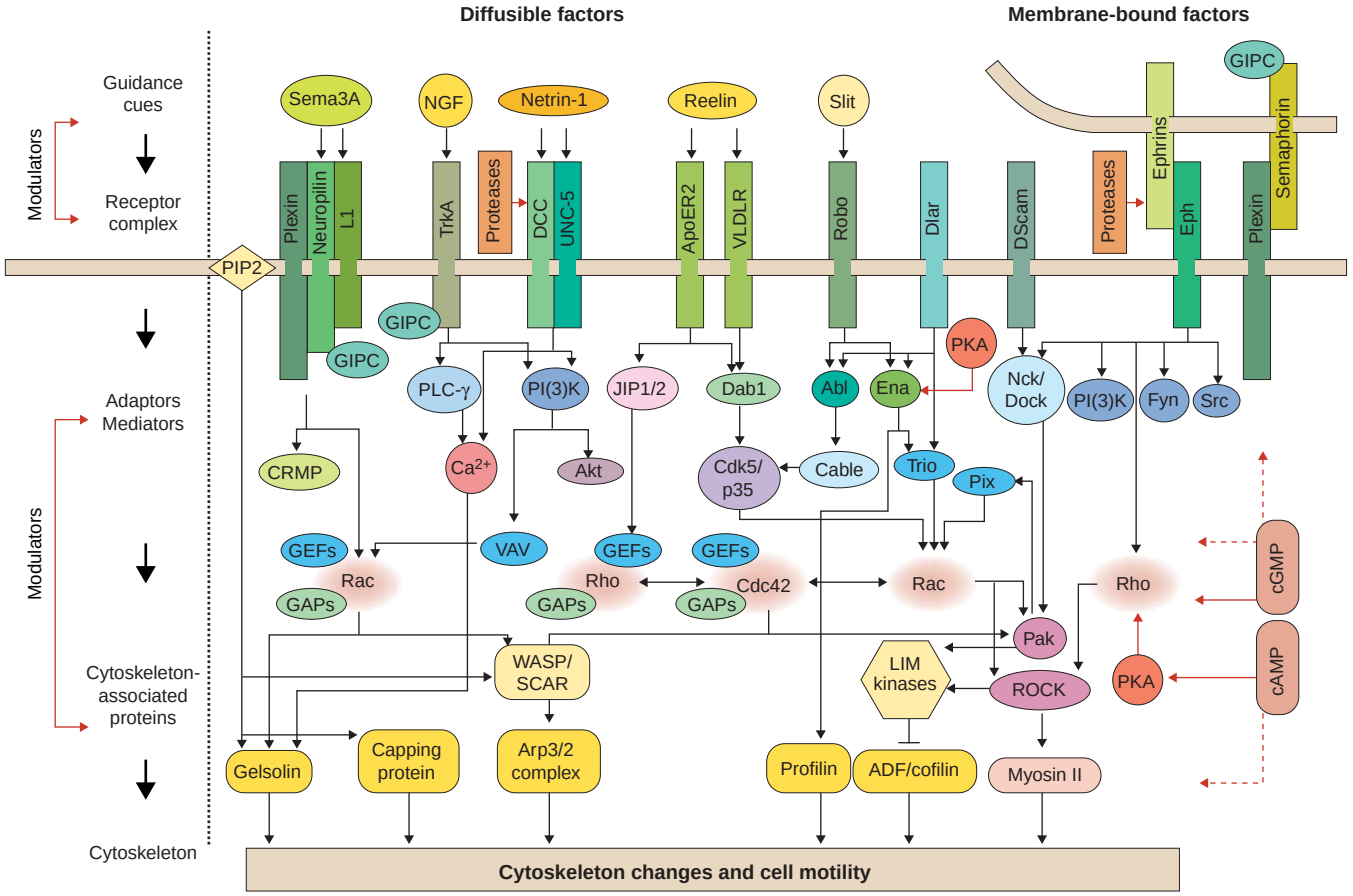

Figure 2 **The interwoven network of signalling molecules that link guidance receptors with the cytoskeletal rearrangement underlying directed neuronal motility.** Various membrane receptors for extracellular guidance cues may function either alone or in a complex to activate cytoplasmic adaptors and mediators. The Rho family of GTPases may be pivotal links between guidance signals and actin-associated proteins, which are responsible for regulating the assembly and disassembly of actin filaments. Similar types of molecules are represented by symbols of similar colour and shape. Lines depict activation pathways that have been demonstrated experimentally in different systems. References for guidance cues, their receptors and cytoplasmic signalling molecules can be obtained from the authors on request.

glycoprotein (MAG) were found to be abolished in the presence PI(3)K inhibitors[54]. In PI(3)K knockout mice, chemotaxis of neutrophils toward chemokines was reduced[75]. One of the immediate downstream targets of PI(3)K is protein kinase B (PKB)/Akt, a PH-domain containing protein. A fluorescently tagged PH domain from Akt was found to translocate to the leading front of chemotaxing neurophils and amoebae[65,66], suggesting that a gradient of PtdIns(3,4,5)P_3 (the product of PI(3)K) is generated in the plasma membrane. In *Dictyostelium* amoebae, PKB/Akt also translocates to the leading front of the migrating cell upon stimulation by chemoattractant[66]. Activation of both PI(3)K and PKB/Akt are required for chemotaxis of these cells. As PI(3)K-related events appear spatially and temporally close to the receptor activation, this enzyme may be pivotal in linking receptors to their downstream effectors.

A group of potential mediators of particular interest is the Rho family of small guanosine triphosphatases (GTPases), including Rho, Rac and Cdc42, that may link many cytoplasmic effectors to the actin cytoskeleton[76], an essential component for cell motility. Rho regulates actin stress fibre formation and focal adhesion, whereas Rac1 and Cdc42 regulate the formation of lamellipodia and filopodia, respectively. Expression of constitutive or dominant-negative forms of Rac1 and Cdc42 cause defects in axon guidance and cell migration in *Caenorhabditis elegans, Drosophila*, mouse and amoebae[77]. Many regulators and downstream effectors of this family of proteins have been identified, but their precise roles in axonal guidance are unclear. Among various guanine nucleotide exchange factors identified for the Rho GTPases, Trio has recently emerged as a key factor for axon guidance[78]. As shown in Fig. 2, these Rho GTPases may integrate many signalling pathways by their potential interactions with many cellular components. As there is no direct evidence as yet that a gradient of their activity exists during directed cell motility, however, these GTPases may be permissive factors for cytoskeleton reorganization rather than direct mediators of guidance signals. Interestingly, introduction of dominant-negative Cdc42 into macrophages results in a reduction of cell polarity and of chemotaxis towards attractants without any substantial effect on cell motility[79], indicating that Cdc42 may be required for transduction of directional signals.

Much is known about proteins that regulate cytoskeleton reorganization[80], which is required for the execution of navigational behaviours[81,82]. Directed motility can be achieved by preferential assembly of actin filaments at the leading front of the cell[83]. It is now increasingly clear that the Arp2/3 complex[80], a group of seven proteins including actin-related proteins Arp2 and Arp3, regulates the assembly of new networks of actin filaments at the leading edges of the migrating cells. Proteins of the Wiskott–Aldrich syndrome protein (WASP) family—WASP, N-WASP, Scars—bind

directly to the Arp2/3 complex and stimulate its ability to promote the nucleation of new actin filaments[80,84]. Members of the Ena/VASP family also colocalize with the Arp2/3 complex at the leading edge of the cells, and localize to the tip of growth cone filopodia[85]. They interact with profilin and catalyse the elongation of newly formed filaments through interaction with monomers and filaments[86].

Capping proteins and gelsolin regulate the growth of actin filaments by regulating the termination of elongation[80]. They also mediate associations between actin and the plasma membrane, and may promote or permit filament elongation under the control of membrane phospholipids. For directed cell motility, it remains unclear how the guidance signal is conveyed from the membrane receptor to the preferential assembly of actin filaments. As most of these actin-associated proteins are potential targets of the Rho family of GTPases, the latter may provide the critical link between the receptor and the cytoskeleton.

Modulating the gain in signal transduction

Neuronal sensitivity to guidance cues can be regulated by other extracellular signals that modulate the level or properties of membrane receptors and cytoplasmic signalling components, which leads to changes in the navigational behaviour (heterologous modulation). Alternatively, guidance cues themselves can trigger changes in the neuron that alters the properties of signal transduction (homologous modulation), as exemplified by neuronal adaptation to different basal level of guidance cues. Although cytoplasmic mechanisms underlying these processes of gain control are largely unexplored, their existence and importance for neuronal navigation are well established.

For chemotaxis of leukocytes, the presence of one attractant causes heterologous desensitization of the cell's response to another attractant[87]. Similarly, the response of neuronal growth cone to a gradient of one guidance cue can be reduced or abolished by the uniform presence of another guidance cue[54]. Modulatory factors may also trigger changes in the neuronal sensitivity to the guidance cue by changing the level and properties of functional receptors. For example, *Drosophila* commissural axons that have crossed the midline upregulate Robo receptors for the midline repellent Slit, in response to the presence of Comm on midline glia surface[88]. Alternatively, secreted proteases can downregulate functional receptors for guidance cues, as shown by the metalloprotease-dependent shedding of DCC in commissural axons[89].

Modulation of cytoplasmic signals can also change the sensitivity to guidance cues. In a gradient of subthreshold (insufficient for chemoattraction) concentrations of netrin-1 or BDNF, the growth cone of *Xenopus* spinal neurons exhibits marked attractive turning after elevation of cytosolic cyclic AMP[59]. In addition to second messengers, adaptors of the guidance cue receptor also can modulate the gain of signal transduction. In *Drosophila* neurons, a mutation in a conserved cytoplasmic tyrosine that can be phosphorylated by adaptor protein Abl generates a hyperactive Robo receptor, which mediates repulsive signals induced by Slit at the midline[90]. In *C. elegans*, migration of Q neuroblasts and their descendants along the anteroposterior body axis is dose-dependent on the level of a transmembrane protein MIG-13 (ref. 91).

In cell cultures, reducing the concentration of substratum-bound laminin (post-translationally) upregulates the amount of $\alpha 6\beta 1$ integrin expressed on the surface of dorsal root ganglion neurons[92], suggesting that in neurons there is a compensatory mechanism for adjusting the level of contact-mediated signals. In a gradient of repellent tectal membrane (or ephrin A2 and A5), the axons of retinal ganglion cells can adjust their sensitivity for different basal concentrations of repellents, so that they grow uphill for a fixed increment of concentration[53]. In the presence of a high basal concentration of guidance cues, the growth cone of *Xenopus* spinal neurons loses its sensitivity towards the gradient of netrin-1 and BDNF, but regains its sensitivity with time and exhibits further chemotropic response even in the presence of the same high basal level of these cues (G. Ming and M-m.P., unpublished observations). When exposed to a gradient of netrin-1 or BDNF, a rapidly extending *Xenopus* growth cone exhibits a 'zigzag' path of alternating attractive and repulsive turning as it migrates up the gradient (Fig. 2), consistent with the existence of a cyclic desensitization by and resensitization to increasingly higher concentrations of guidance cues.

In bacteria chemotaxis, the steady-state level of receptor methylation provided by the opposing methytransferase and methylesterase reactions enables the signalling pathway to adapt to the basal level of stimuli and allows the bacteria to chemotax in a concentration gradient superimposed on a wide range of constant level of attractant or repellent[93]. A similar mechanism of receptor modulation may also account for neuronal adaptation, although adaptive modulation of downstream effectors may also occur.

Modulation of the polarity of navigation

Most guidance cues identified so far are bi-functional: they are attractants or repellents for different types of neurons, as well as for different developmental states or different parts (dendrites versus axon) of the same neuron. Here we discuss potential mechanisms by which the neuron determines the polarity—attraction versus repulsion—in its behaviour towards the same guidance cue.

Depending on the availability of different receptors to the same guidance cues, the growth cone can have exhibit opposite navigational behaviours. For example, in *C. elegans* ectopic expression of UNC-5 in touch neurons converted UNC-6 (netrin-1)-dependent migration from a ventral to a dorsal direction[94]. Similarly, growth cones of cultured *Xenopus* spinal neurons exhibited DCC-dependent attraction in a netrin-1gradient, but showed repulsive response in the same gradient when the neuron overexpressed UNC-5 protein[44], a switch that depends on the netrin-1-triggered interaction between the cytoplasmic domains of DCC and UNC-5.

The cytoplasmic domain of the receptor is critical in determining the guidance behaviour; and this has been nicely shown by the finding that exchanging the cytoplasmic domain of frazzel (a *Drosophila* netrin receptor) with that of Robo converts netrin-A-induced attraction to repulsion, and vice versa[95]. It is possible that the cytoplasmic domain of some guidance cue receptors can activate both attractive and repulsive signalling pathways; interaction of or enzymatic modification of receptors/adaptors selectively activate or inhibit one of the two pathways.

Depending on the levels of cytoplasmic cAMP/cGMP, the response of the growth cone to a guidance cue can be converted between attraction and repulsion. Studies of cultured *Xenopus* spinal neurons have shown that all guidance cues examined for these neurons can be classified into two groups[43]. In group I, which includes netrin-1, BDNF, ACh and MAG, the level of cytosolic cAMP or the activity of protein kinase A (PKA) is critical in determining whether the turning response is attractive or repulsive. Inhibition of PKA converts attraction induced by a gradient of netrin-1, BDNF or ACh into repulsion, whereas activation of PKA converts repulsion induced by MAG into attraction. In group II, which includes Sema 3A and NT-3, the turning response is regulated by cGMP or protein kinase G (PKG)[60]. Activation of PKG converts repulsive turning induced by Sema 3A into attraction, whereas inhibition of PKG converts NT-3-induced attraction into repulsion.

Interestingly, the polarity of the growth cone behaviour can also be regulated separately within dendritic/axonal compartments of the same neuron. In developing cortex, Sema3A secreted by cells in the marginal zone seems to repel axons of developing cortical neurons but attracts their apical dendrites, resulting in a polarized projection of axons and dendrites in the cortex[45]. The attractive guidance of apical dendrites can be abolished by reducing cGMP

activity in the neuron, and an asymmetric localization of soluble guanylate cyclase seems to confer the bipolar responses of the neuron towards Sema 3A[45]. The repulsive response of dorsal root ganglion and cortical axons towards Sema3A can also be converted into attraction by exposure to soluble LIFc chimaeric molecules and activation of guanylate cyclase activity is required for the conversion[69]. The targets of PKA/PKG that are involved in regulating the polarity of navigation remain to be identified. Notably, the Ena/VASP family of proteins, which are implicated in controlling the actin dynamics[85], are substrates of PKA/PKG.

Phosphorylation-dependent polymerization/depolymerization can, in principle, reverse the direction of polarized cytoskeleton assembly at the leading front, resulting in a change in the polarity of neuronal navigation[43]. Many extracellular ligands, including neuromodulators, adhesion molecules and ECM components, can change the level of cyclic nucleotides and are thus capable of modulating axon navigation when they are present concurrently with the guidance cue. For example, exposure of cultured *Xenopus* retinal ganglion cells to laminin causes a conversion of their growth cone responses in a netrin-1 or BDNF gradient from attraction to repulsion, through downregulating the cytoplasmic level of cAMP[23].

The polarity of navigation may be reset by gene regulation and protein synthesis. The direction of the migration of Q neuroblasts and their descendents along the anteroposterior body axis in *C. elegans* is determined by the expression of the Hox gene *mab-5*, which in turn is controlled by a Wnt signalling pathway[96]. Further elucidation of the cellular actions of Mab-5 protein and its downstream effectors may reveal how the polarity of neuronal migration is regulated.

Concluding remarks

Despite remarkable progress in the molecular identification of guidance molecules, their receptors and the components of signal transduction pathways, there remain big gaps in our understanding of the cell biology of neuronal navigation. There are the immediate tasks of identifying the missing links in the signalling cascades and distinguishing true mediators of guidance signals from a myriad of permissive/modulatory factors. These tasks are likely to be greatly facilitated by comparison with the signalling events that have been identified for the chemotaxis of non-neuronal cells. For neuronal navigation as well as for directed cell migration in general, major challenges lie in elucidating the spatiotemporal regulation of interwoven signalling pathways; such regulation allows the cell to amplify the guidance signal and to adapt to the changing concentration of guidance signals in the environment.

Neuronal navigation exemplifies the intelligent behaviour of single cells. Migrating neurons and growth cones resemble unicellular organisms interacting with its environment. This cellular intelligence is derived from the adaptive properties of a network of signal transduction pathways, which are analogous to the neural networks that form the basis of adaptive learning systems[97]. Complementary to the molecular dissection of signalling pathways, a computational approach to the analysis of interwoven networks of signalling molecules[98], with a focus on the input–output relationship of various components in the network and their role in the amplification and adaptation of guidance signals, may be necessary for an integrative understanding of the cell biology of neuronal navigation. □

1. Kandel, E., Schwartz, J. & Jessel, T. M. *Principles of Neural Sciences* 4th edn (McGraw-Hill, New York, 2000).
2. Sane, D. H., Reh, T. A. & Harris, W. A. *Development of the Nervous System* (Academic, San Diego, 2000).
3. Rakic, P. & Lombroso, P. J. Development of the cerebral cortex: I. Forming the cortical structure. *J. Am. Acad. Child Adolesc. Psychiat.* **37,** 116–117 (1998).
4. Hatten, M. E. Central nervous system neuronal migration. *Annu. Rev. Neurosci.* **22,** 511–539 (1999).
5. Le Douarin, N. M., Hallonet, M. E. & Pourquie, O. Cell migrations and establishment of neuronal connections in the developing brain: a study using the quail-chick chimera system. *Prog. Brain Res.* **100,** 3–18 (1994).
6. Goodman, C. S. & Tessier-Lavigne, M. in *Molecular and Cellular Approaches to Neural Development* (eds Cowan, W. M., Jessell, T. M. & Zipursky, S. L.) 108–178 (Oxford Univ. Press, 1997).
7. Devreotes, P. N. & Zigmond, S. H. Chemotaxis in eukaryotic cells: a focus on leukocytes and Dictyostelium. *Annu. Rev. Cell Biol.* **4,** 649–686 (1988).
8. Mueller, B. K. Growth cone guidance: first steps towards a deeper understanding. *Annu. Rev. Neurosci.* **22,** 351–388 (1999).
9. Tessier-Lavigne, M. & Goodman, C. S. The molecular biology of axon guidance. *Science* **274,** 1123–1133 (1996).
10. Harrison, R. G. The reaction of embryonic cells to solid structures. *J. Exp. Zool.* **17,** 521–544 (1914).
11. Weiss, P. A. In vitro experiments on the factors determining the course of the outgrowing nerve fiber. *J. Exp. Zool.* **68,** 393–448 (1934).
12. Holtfreter, J. Neural differentiation of ectoderm through exposure to saline solution. *J. Exp. Zool.* **95,** 307–343 (1944).
13. Sperry, R. W. Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. USA* **50,** 703–710 (1963).
14. Walsh, F. S. & Doherty, P. Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. *Annu. Rev. Cell Dev. Biol.* **13,** 425–456 (1997).
15. Bentley, D. & Caudy, M. Pioneer axons lose directed growth after selective killing of guidepost cells. *Nature* **304,** 62–65 (1983).
16. Bastiani, M. J., Harrelson, A. L., Snow, P. M. & Goodman, C. S. Expression of fasciclin I and II glycoproteins on subsets of axon pathways during neuronal development in the grasshopper. *Cell* **48,** 745–755 (1987).
17. Zheng, C., Heintz, N. & Hatten, M. E. CNS gene encoding astrotactin, which supports neuronal migration along glial fibers. *Science* **272,** 417–419 (1996).
18. Anton, E. S., Kreidberg, J. A. & Rakic, P. Distinct functions of alpha3 and alpha(v) integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron* **22,** 277–289 (1999).
19. Dulabon, L. *et al.* Reelin binds α3β1 integrin and inhibits neuronal migration. *Neuron* **27,** 33–44 (2000).
20. Doetsch, F., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* **17,** 5046–5061 (1997).
21. Lallier, T., Deutzmann, R., Perris, R. & Bronner-Fraser, M. Neural crest cell interactions with laminin: structural requirements and localization of the binding site for α1β1 integrin. *Dev. Biol.* **162,** 451–464 (1994).
22. McKenna, M. P. & Raper, J. A. Growth cone behavior on gradients of substratum bound laminin. *Dev. Biol.* **130,** 232–236 (1988).
23. Hopker, V. H., Shewan, D., Tessier-Lavigne, M., Poo, M. & Holt, C. Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. *Nature* **401,** 69–73 (1999).
24. Krull, C. E. *et al.* Interactions of Eph-related receptors and ligands confer rostrocaudal pattern to trunk neural crest migration. *Curr. Biol.* **7,** 571–580 (1997).
25. Hattori, M., Osterfield, M. & Flanagan, J. G. Regulated cleavage of a contact-mediated axon repellent. *Science* **289,** 1360–1365 (2000).
26. Cajal, S. R. in *Histology of the Nervous System* Vol. 1 (Transl. Swanson, N. & Swanson, L. W.) 532–537 (Oxford Univ. Press, New York, 1995).
27. Ebendal, T. & Jacobson, C. O. Tissue explants affecting extension and orientation of axons in cultured chick embryo ganglia. *Exp. Cell Res.* **105,** 379–387 (1977).
28. Tessier-Lavigne, M., Placzek, M., Lumsden, A. G., Dodd, J. & Jessell, T. M. Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature* **336,** 775–778 (1988).
29. Pini, A. Chemorepulsion of axons in the developing mammalian central nervous system. *Science* **261,** 95–98 (1993).
30. Keynes, R. *et al.* Surround repulsion of spinal sensory axons in higher vertebrate embryos. *Neuron* **18,** 889–897 (1997).
31. Lumsden, A. G. & Davies, A. M. Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor. *Nature* **306,** 786–788 (1983).
32. Harris, W. A. Homing behaviour of axons in the embryonic vertebrate brain. *Nature* **320,** 266–269 (1986).
33. Brose, K. & Tessier-Lavigne, M. Slit proteins: key regulators of axon guidance, axonal branching, and cell migration. *Curr. Opin. Neurobiol.* **10,** 95–102 (2000).
34. Harris, W. A. Local positional cues in the neuroepithelium guide retinal axons in embryonic *Xenopus* brain. *Nature* **339,** 218–221 (1989).
35. Nakamura, H. & O'Leary, D. D. Inaccuracies in initial growth and arborization of chick retinotectal axons followed by course corrections and axon remodeling to develop topographic order. *J. Neurosci.* **9,** 3776–3795 (1989).
36. Trowe, T. *et al.* Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, Danio rerio. *Development* **123,** 439–450 (1996).
37. Gierer, A. Model for the retino-tectal projection. *Proc. R. Soc. Lond. B* **218,** 77–93 (1983).
38. Loschinger, J., Weth, F. & Bonhoeffer, F. Reading of concentration gradients by axonal growth cones. *Phil. Trans. R. Soc. Lond. B* **355,** 1–12 (2000).
39. Walsh, C. A. & Goffinet, A. M. Potential mechanisms of mutations that affect neuronal migration in man and mouse. *Curr. Opin. Genet. Dev.* **10,** 270–274 (2000).
40. Tang, J., Rutishauser, U. & Landmesser, L. Polysialic acid regulates growth cone behavior during sorting of motor axons in the plexus region. *Neuron* **13,** 405–414 (1994).
41. Stoeckli, E. T., Sonderegger, P., Pollerberg, G. E. & Landmesser, L. T. Interference with axonin-1 and NrCAM interactions unmasks a floor-plate activity inhibitory for commissural axons. *Neuron* **18,** 209–221 (1997).
42. Jurata, L. W., Thomas, J. B. & Pfaff, S. L. Transcriptional mechanisms in the development of motor control. *Curr. Opin. Neurobiol.* **10,** 72–79 (2000).
43. Song, H. J. & Poo, M. M. Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.* **9,** 355–363 (1999).
44. Hong, K. *et al.* A ligand-gated association between cytoplasmic domains of UNC5 and DCC family

receptors converts netrin-induced growth cone attraction to repulsion. *Cell* **97,** 927–941 (1999).
45. Polleux, F., Morrow, T. & Ghosh, A. Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature* **404,** 567–573 (2000).
46. Blochl, A. & Thoenen, H. Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *Eur. J. Neurosci.* **7,** 1220–1228 (1995).
47. Wang, X., Berninger, B. & Poo, M. Localized synaptic actions of neurotrophin-4. *J. Neurosci.* **18,** 4985–4992 (1998).
48. Halloran, M. C. *et al.* Laser-induced gene expression in specific cells of transgenic zebrafish. *Development* **127,** 1953–1960 (2000).
49. Yee, K. T., Simon, H. H., Tessier-Lavigne, M. & O'Leary, D. M. Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1. *Neuron* **24,** 607–622 (1999).
50. Hu, H. Chemorepulsion of neuronal migration by Slit2 in the developing mammalian forebrain. *Neuron* **23,** 703–711 (1999).
51. Wu, W. *et al.* Directional guidance of neuronal migration in the olfactory system by the protein Slit. *Nature* **400,** 331–336 (1999).
52. Zhu, Y., Li, H., Zhou, L., Wu, J. Y. & Rao, Y. Cellular and molecular guidance of GABAergic neuronal migration from an extracortical origin to the neocortex. *Neuron* **23,** 473–485 (1999).
53. Rosentreter, S. M. *et al.* Response of retinal ganglion cell axons to striped linear gradients of repellent guidance molecules. *J. Neurobiol.* **37,** 541–562 (1998).
54. Ming, G. *et al.* Phospholipase C-γ and phosphoinositide 3-kinase mediate cytoplasmic signaling in nerve growth cone guidance. *Neuron* **23,** 139–148 (1999).
55. Goodhill, G. J. & Urbach, J. S. Theoretical analysis of gradient detection by growth cones. *J. Neurobiol.* **41,** 230–241 (1999).
56. Zigmond, S. H. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J. Cell Biol.* **75,** 606–616 (1977).
57. Lohof, A. M., Quillan, M., Dan, Y. & Poo, M. M. Asymmetric modulation of cytosolic cAMP activity induces growth cone turning. *J. Neurosci.* **12,** 1253–1261 (1992).
58. Zheng, J. Q., Felder, M., Connor, J. A. & Poo, M. M. Turning of nerve growth cones induced by neurotransmitters. *Nature* **368,** 140–144 (1994).
59. Song, H. J., Ming, G. L. & Poo, M. M. cAMP-induced switching in turning direction of nerve growth cones. *Nature* **388,** 275–279 (1997).
60. Song, H. J. *et al.* Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. *Science* **281,** 1515–1518 (1998).
61. Baier, H. & Bonhoeffer, F. Axon guidance by gradients of a target-derived component. *Science* **255,** 472–475 (1992).
62. Meinhardt, H. Orientation of chemotactic cells and growth cones: models and mechanisms. *J. Cell Sci.* **112,** 2867–2874 (1999).
63. Nieto, M. *et al.* Polarization of chemokine receptors to the leading edge during lymphocyte chemotaxis. *J. Exp. Med.* **186,** 153–158 (1997).
64. Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B. & Devreotes, P. N. G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* **95,** 81–91 (1998).
65. Servant, G. *et al.* Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. *Science* **287,** 1037–1040 (2000).
66. Firtel, R. A. & Chung, C. Y. The molecular genetics of chemotaxis: sensing and responding to chemoattractant gradients. *BioEssays* **22,** 603–615 (2000).
67. Goldberg, D. J. & Wu, D. Y. Tyrosine phosphorylation and protrusive structures of the growth cone. *Perspect. Dev. Neurobiol.* **4,** 183–192 (1996).
68. Nakamura, F., Kalb, R. G. & Strittmatter, S. M. Molecular basis of semaphorin-mediated axon guidance. *J. Neurobiol.* **44,** 219–229 (2000).
69. Castellani, V., Chedotal, A., Schachner, M., Faivre-Sarrailh, C. & Rougon, G. Analysis of the L1-deficient mouse phenotype reveals cross-talk between Sema3A and L1 signaling pathways in axonal guidance. *Neuron* **27,** 237–249 (2000).
70. Gilmore, E. C. & Herrup, K. Cortical development: receiving reelin. *Curr. Biol.* **10,** R162–R166 (2000).
71. Ming, G. L. *et al.* cAMP-dependent growth cone guidance by netrin-1. *Neuron* **19,** 1225–1235 (1997).
72. Hong, K., Nishiyama, M., Henley, J., Tessier-Lavigne, M. & Poo, M. Calcium signalling in the guidance of nerve growth by netrin-1. *Nature* **403,** 93–98 (2000).
73. Zheng, J. Q. Turning of nerve growth cones induced by localized increases in intracellular calcium ions. *Nature* **403,** 89–93 (2000).
74. Rakic, P. & Komuro, H. The role of receptor/channel activity in neuronal cell migration. *J. Neurobiol.* **26,** 299–315 (1995).
75. Wymann, M. P., Sozzani, S., Altruda, F., Mantovani, A. & Hirsch, E. Lipids on the move: phosphoinositide 3-kinases in leukocyte function. *Immunol. Today* **21,** 260–264 (2000).
76. Bar-Sagi, D. & Hall, A. Ras and Rho GTPases: A familiy reunion. *Cell* **103,** 227–238(2000).
77. Luo, L. Rho GTPases in neuronal morphogenesis. *Nature Rev. Neurosci.* **1,** 173–180 (2000).
78. Newsome, T. P. *et al.* Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in *Drosophila. Cell* **101,** 283–294 (2000).
79. Allen, W. E., Zicha, D., Ridley, A. J. & Jones, G. E. A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* **141,** 1147–1157 (1998).
80. Pollard, T. D., Blanchoin, L. & Mullins, R. D. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu. Rev. Biophys. Biomol. Struct.* **29,** 545–576 (2000).
81. Mitchison, T. J. & Cramer, L. P. Actin-based cell motility and cell locomotion. *Cell* **84,** 371–379 (1996).
82. Suter, D. M. & Forscher, P. Substrate-cytoskeletal coupling as a mechanism for the regulation of growth cone motility and guidance. *J. Neurobiol.* **44,** 97–113 (2000).
83. Weiner, O. D. *et al.* Spatial control of actin polymerization during neutrophil chemotaxis. *Nature Cell Biol.* **1,** 75–81 (1999).
84. Rohatgi, R. *et al.* The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97,** 221–231 (1999).
85. Lanier, L. M. & Gertler, F. B. From Abl to actin: Abl tyrosine kinase and associated proteins in growth cone motility. *Curr. Opin. Neurobiol.* **10,** 80–87 (2000).
86. Laurent, V. *et al.* Role of proteins of the Ena/VASP family in actin-based motility of Listeria monocytogenes. *J. Cell Biol.* **144,** 1245–1258 (1999).
87. Ali, H., Richardson, R. M., Haribabu, B. & Snyderman, R. Chemoattractant receptor cross-desensitization. *J. Biol. Chem.* **274,** 6027–6030 (1999).
88. Kidd, T., Russell, C., Goodman, C. S. & Tear, G. Dosage-sensitive and complementary functions of roundabout and commissureless control axon crossing of the CNS midline. *Neuron* **20,** 25–33 (1998).
89. Galko, M. J. & Tessier-Lavigne, M. Function of an axonal chemoattractant modulated by metalloprotease activity. *Science* **289,** 1365–1367 (2000).
90. Bashaw, G. J., Kidd, T., Murray, D., Pawson, T. & Goodman, C. S. Repulsive axon guidance: Abelson and Enabled play opposing roles downstream of the roundabout receptor. *Cell* **101,** 703–715 (2000).
91. Sym, M., Robinson, N. & Kenyon, C. MIG-13 positions migrating cells along the anteroposterior body axis of *C. elegans. Cell* **98,** 25–36 (1999).
92. Condic, M. L. & Letourneau, P. C. Ligand-induced changes in integrin expression regulate neuronal adhesion and neurite outgrowth. *Nature* **389,** 852–856 (1997).
93. Falke, J. J., Bass, R. B., Butler, S. L., Chervitz, S. A. & Danielson, M. A. The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Annu. Rev. Cell Dev. Biol.* **13,** 457–512 (1997).
94. Hamelin, M., Zhou, Y., Su, M. W., Scott, I. M. & Culotti, J. G. Expression of the UNC-5 guidance receptor in the touch neurons of *C. elegans* steers their axons dorsally. *Nature* **364,** 327–330 (1993).
95. Bashaw, G. J. & Goodman, C. S. Chimeric axon guidance receptors: the cytoplasmic domains of slit and netrin receptors specify attraction versus repulsion. *Cell* **97,** 917–926 (1999).
96. Branda, C. S. & Stern, M. J. Cell migration and axon growth cone guidance in *Caenorhabditis elegans. Curr. Opin. Genet. Dev.* **9,** 479–484 (1999).
97. Bray, D. Protein molecules as computational elements in living cells. *Nature* **376,** 307–312 (1995).
98. Jodan, J. D., Landau, E. M., & Iyengar, R. Signaling networks: the origins of cellular multitasking. *Cell* **103,** 193–200 (2000).
99. Anand-Apte, B. & Zetter, B. Signaling mechanisms in growth factor-stimulated cell motility. *Stem Cells* **15,** 259–267 (1997).
100. Sanchez-Madrid, F. & del Pozo, M. A. Leukocyte polarization in cell migration and immune interactions. *EMBO J.* **18,** 501–511 (1999).

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