Signal transduction underlying growth cone guidance by diffusible factors

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Many diffusible axon guidance cues and their receptors have been identified recently. These cues are often found to be bifunctional, acting as attractants or repellents under different circumstances. Studies of cytoplasmic signaling mechanisms have led to the notion that the response of a growth cone to a particular guidance cue depends on the internal state of the neuron, which, in turn, is under the influence of other coincident signals received by the neuron. Furthermore, many diffusible guidance cues appear to share common cytoplasmic signaling pathways.

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Abbreviations

ACh	acetylcholine
BDNF	brain-derived trophic factor
CaM	calmodulin
CAM	cell adhesion molecule
DCC	Deleted in Colorectal Cancer
DD	death domain
Dlg	Discs large
DRG	dorsal root ganglion
ECM	extracellular matrix
FGF	fibroblast growth factor
GTPase	guanosine triphosphatase
HGF/SF	hepatocyte growth factor/scatter factor
IP_3	inositol 1,4,5-trisphosphate
MAG	myelin-associated glycoprotein
NCAM	neural CAM
NGF	nerve growth factor
NPN	neuropilin
NT-3	neurotrophin-3
PDZ	PSD-95, Dlg and ZO-1
PI-3 kinase	phosphoinositol-3 kinase
PKA	protein kinase A
PKG	protein kinase G
PLC-γ	phospholipase C-γ
PSD-95	postsynaptic density protein of 95 kDa
Robo	roundabout
Sema	semaphorin
Sema III	collapsin-1/Sema III/D
ZO-1	zona occludens 1

Introduction

The development of the nervous system depends on correct pathfinding and target recognition by the growing tip of an axon, the growth cone. Diffusible or substrate-bound molecules present in the environment may serve as either attractants or repellents to influence the direction of growth cone extension (see [1,2••] for reviews). A guidance cue interacts with specific receptor molecules on the axonal surface of the growth cone and triggers a cascade of cytoplasmic events that eventually leads to the cytoskeletal rearrangement associated with oriented neurite extension. Many factors have been proposed as potential diffusible guidance cues in different systems. In some cases, such as netrins and semaphorins [1,2••], the physiological guidance function has been demonstrated extensively both *in vivo* and *in vitro*. In other cases, such as neurotrophins, guidance function has only been shown clearly *in vitro* [3–5].

Studies performed in the past two decades have provided detailed descriptions of growth cone behaviors in a variety of *in vitro* and *in vivo* environments. Our understanding of the molecular mechanisms underlying growth cone behavior, however, is much more fragmentary. Recent biochemical and genetic studies have led to the identification of several families of guidance cues and their receptors (for reviews, see [1,2••]). In this review, we first summarize recent advances in the identification of a few evolutionarily conserved ligand–receptor systems relevant to growth cone guidance. This is followed by a more detailed description of cytoplasmic signal transduction for these systems. A number of comprehensive reviews on axonal guidance with different emphases have appeared recently [1,2••,6–10].

Guidance cues and their receptors

Netrins – DCC and UNC-5

The netrins constitute a small family of proteins with phylogenetically conserved functions in axon guidance [9]. They are secreted molecules related to laminin, a molecule associated with the extracellular matrix (ECM). *In vitro*, netrins can induce attractive or repulsive responses in different neurons depending on the type of receptors expressed [11,12] and in the same neuron depending on the intracellular level of cAMP [13•]. Distinct and complimentary evidence from worms, flies and vertebrates has implicated the involvement of netrins in axon guidance *in vivo* [1,2••,9].

The signal transduction mechanisms by which netrins produce attractive and repulsive actions are still poorly understood. In the case of chemoattraction, Deleted in Colorectal Cancer (DCC) in rodents and its homologs UNC-40 and Frazzled in *Caenorhabditis elegans* and *Drosophila*, respectively [14–16], have been identified as the membrane receptor. It is not known whether each of these DCC-related proteins constitutes the entire receptor involved in the attractive response. The immediate downstream effectors of DCC are unknown. However, mammalian homologs of Seven in Absentia have been

shown to bind the cytoplasmic domain of DCC and to regulate DCC via the ubiquitin-proteasome pathway [17].

In the case of netrin-mediated repulsion, studies of *C. elegans* have implicated UNC-5 as a netrin receptor or a component of it [11,12]. In addition, vertebrate homologs of UNC-5 (i.e. UNC-5H1, UNC-5H2 and UNC-5H3) bind netrin [18]. Additional evidence suggests that DCC may be part of a repulsive receptor complex: netrin homolog (UNC-6)-induced repulsion shows some dependence on the function of UNC-40 in *C. elegans* [11]. Furthermore, both attraction and repulsion of *Xenopus* spinal neurons in response to netrin-1 require DCC [13•]. Recently, UNC-5 has been shown to interact directly with DCC through its cytoplasmic domain (K Hong, E Stein, M-m Poo, M Tessier-Lavigne, unpublished observations). Furthermore, expression of exogenous UNC-5 (or its homologs) in cultured *Xenopus* spinal neurons can switch the attraction induced by a netrin-1 gradient to repulsion, and this switch depends on the interaction between the cytoplasmic domains of DCC and UNC-5.

The downstream effectors of UNC-5 are presently unknown. Within the cytoplasmic domain of UNC-5, there is a death domain (DD), which is known to exhibit homophilic DD–DD interactions. Thus, UNC-5 has the potential to interact with proteins containing a DD domain, such as ankyrin, a spectrin-binding protein that links integral membrane proteins to the underlying cytoskeleton [19]. In *C. elegans* suppressors of growth cone steering induced by ectopic UNC-5 include four new genes as well as four genes previously known to be required for axon guidance, *unc-6*, *unc-40*, *unc-44* and *unc-34* [20]. Interestingly, *unc-44* encodes an ankyrin-related protein, thus providing a putative link between UNC-5 and the cytoskeleton. Homologs of UNC-34, Mena and Abl, have been found to be profilin-binding proteins and required for axon guidance in mice and *Drosophila*, respectively [21–23].

Semaphorins – neuropilins and plexins

The semaphorins are a large family of secreted and transmembrane proteins that are conserved phylogenetically [1,2••,10,24]. Many members of this family can serve as repulsive guidance cues, influencing axonal pathfinding as a bound factor or as a diffusible chemorepellent. There are at least eight different subtypes, including viral semaphorins [24]. Different semaphorin family members can provide functional distinct guidance information to the growth cone [25]. Class III semaphorins — Sema A, collapsin-1/Sema III/D (Sema III), and Sema E — are secreted and synthesized as pro-proteins, whose activity is regulated by proteolytic processes [26]. In vertebrates, Sema III can function as a potent chemorepellent both *in vitro* and *in vivo*; other class III semaphorins probably function in a similar fashion [1,2••]. On the other hand, Sema III and Sema E can also function as attractive guidance cues *in vitro* [27••,28•].

Neuropilins have recently been identified as receptors for semaphorins [29••,30••,31•]. Sema III binds with high affinity to neuropilin-1 (NPN-1). Mice deficient in NPN-1 display severe abnormalities in the trajectory of efferent fibers of the peripheral nervous system, and the growth cones of dorsal root ganglion (DRG) neurons from these mutant mice do not collapse in the presence of Sema III [32]. Neuropilin-2 (NPN-2), a homolog of NPN-1, binds with high affinity to Sema E and Sema IV but not to Sema III, whereas NPN-1 binds with high affinity to all three of these semaphorins [31•,33•]. Sema III and Sema IV signaling appear to be mediated predominately by NPN-1 and NPN-2 oligomers, respectively, whereas Sema E signaling appears to be mediated by both NPN-1 and NPN-2 as homo- or hetero-oligomers [33•,34,35•]. The cytoplasmic domain of NPN-1 is small, about 40 amino acids in length, with no recognizable motif, and it is dispensable for the semaphorin-induced repulsive response [36•]. Thus, NPN-1 may serve an essential role for assembling a receptor complex that includes other unknown components. We (Z He, H-j Song, M-m Poo, M Tessier-Lavigne, unpublished observations) have recently identified a PDZ-domain-containing NPN-associated protein using yeast two-hybrid screening and showed that it is required for Sema-III-induced repulsion in *Xenopus* spinal neurons. Two proteins, collapsin-response-mediator-protein [37] and Rac1 [38,39], have also been implicated as downstream signaling components, but how they are linked to NPN or the NPN-associated complex is unknown.

In addition to NPNs, recent studies have identified a different family of transmembrane proteins named plexins, some of which can serve as receptors for class I and V semaphorins [40,41•]. It is possible that there are more functional plexin–semaphorin pairs in the nervous system.

Slit – robo

Recent studies in *Drosophila* have revealed that a transmembrane protein encoded by the *roundabout* (*robo*) gene functions as a receptor to a midline repellent to ensure no inappropriate crossing of the midline [7,42••]. High-level expression of Robo on growth cones prevents midline crossing. Commissural axons express a high level of Robo only after they have crossed the midline. The presence of another novel membrane protein, commissureless, is responsible for the downregulation of Robo at the midline [43,44]. A *C. elegans* homolog of Robo, Sax-3, appears to serve a similar function in axonal guidance near the midline [45••].

The ligand for Robo has been identified as Slit, an extracellular matrix protein secreted by midline glial cells [46••–48••]. In *Drosophila*, mutations of Slit result in the collapse of the regular scaffold of commissural and longitudinal axon tracts in the embryonic central nervous system [49]. Genetic interactions between Robo and Slit suggests that these two molecules act in the same pathway [46••]. *In vitro*, Slit binds with high affinity to

Robo on the cell surface [47••,48••], and acts as a chemorepellent for axons from olfactory bulb, embryonic spinal cord and hippocampal explants [47••,48••,50]. Interestingly, Slit also promotes branching and growth of sensory axons *in vitro* [51].

Growth factors – receptor tyrosine kinases

Growth factors have long been implicated in axon guidance since the early finding that injection of nerve growth factor (NGF) into neonatal rodent brains causes aberrant growth of peripheral sympathetic axons to the site of injection [52]. Substratum-bound NGF and gradients of soluble NGF can direct elongating DRG axons *in vitro* [3,4]. A gradient of brain-derived trophic factor (BDNF) or neurotrophin 3 (NT-3) attracts the growth cone of cultured *Xenopus* spinal neurons [5,53••]. In NT-3 deficient mice, sympathetic neurons fail to invade the pineal gland and external ear (tissues that secrete NT-3), a defect that can be rescued by the addition of exogenous NT-3 [54]. Neurotrophins may also function as chemorepellents. Cultured *Xenopus* spinal neurons are chemorepulsed in a BDNF or NT-3 gradient when cAMP or cGMP signaling are inhibited, respectively [27••,53••]. For young spinal neurons in these cultures, BDNF causes growth cone collapse, which can be prevented by elevating cAMP signaling [55]. Neurotrophins bind two receptor types – a shared low-affinity receptor p75 and ligand-specific receptor tyrosine kinases of the Trk family [56]. Activation of Trk triggers multiple intracellular signaling pathways, including the RAS-MAP kinase pathway, the phospholipase C-γ (PLC-γ) and phosphoinositol-3 kinase (PI-3 kinase) pathway.

In addition to neurotrophins, other growth factors may also act as attractants and repellents. Hepatocyte growth factor/scatter factor (HGF/SF) has been found to be a survival factor for motor neurons and an attractant for their axons [57]. The c-Met receptor tyrosine kinase family mediates the actions of HGF/SF. Fibroblast growth factor (FGF) stimulates retinal neurite extension *in vitro* and causes mistargeting of retinal axons when applied exogenously to the developing optic projection [58]. A similar aberrant targeting has been observed when FGF signaling in the growth cone was blocked by the expression of a dominant-negative FGF receptor in retinal ganglion cells [59]. Recently, it has been shown that motor axons along the dorsal–ventral axis of *C. elegans* are guided by UNC-129, a member of the transforming growth factor-β family [60•].

Other potential guidance cues

In addition to well-established chemoattractants and chemorepellents, there exists a large group of factors that can modulate neurite growth, including adhesion molecules and components of the ECM. It is possible that all these molecules, when presented in a gradient, can function as guidance molecules to influence the direction of axon extension. For example, a gradient of myelin-associated glycoprotein (MAG), an inhibitor of axonal regeneration whose extracellular domain can be released *in vivo* as a soluble fragment [61], has been found to cause repulsive turning of growth cones of cultured *Xenopus* spinal neurons [27••]. Several cell adhesion molecules (including neural CAM, L1, and neural cadherin) have soluble isoforms generated by proteolytic cleavage of a transmembrane form or by shedding of a glycosylphosphophatidyl inositol-anchored form [62]. They can affect nerve growth by modulating cascades of second-messenger systems [62] and may act as diffusible guidance cues if a gradient of these molecules can be established within the tissue.

Cytoplasmic signal transduction

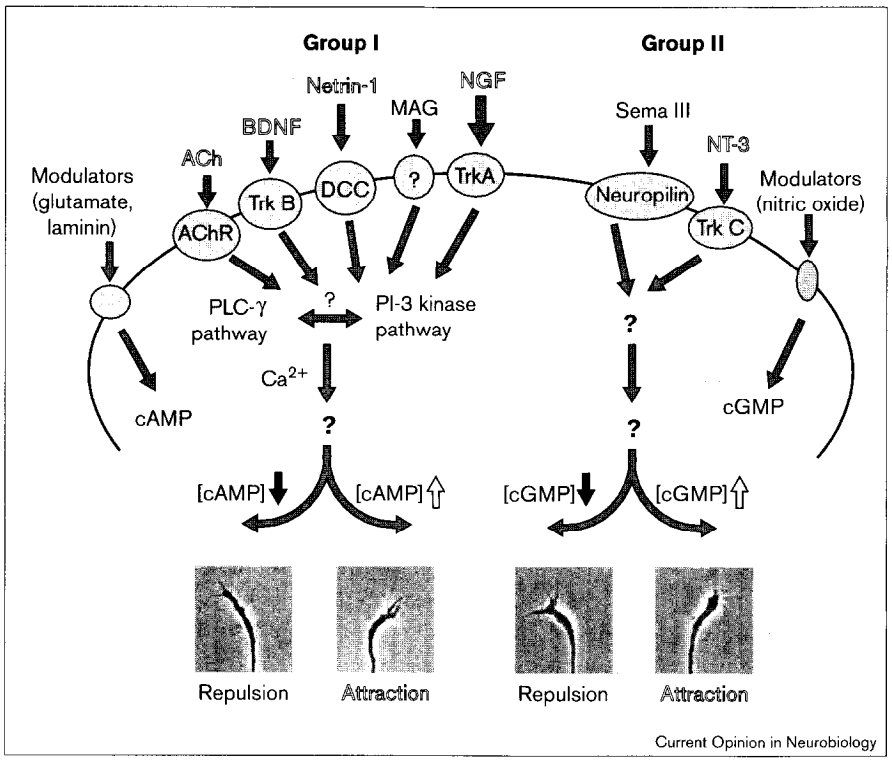
Various in *vitro* and *in vivo* assays have implicated different second-messenger systems as mediators for various aspects of neurite initiation or extension, growth cone collapse or turning [63]. However, the existing information is rather fragmentary and no definitive pictures can be discerned. In the sections below, we attempt to provide an overview of the potential involvement of several key components, with emphasis on the role of cyclic nucleotides in regulating the growth cone responses induced by a variety of guidance cues.

Calcium

Calcium has long been recognized for its role in regulating a wide range of growth cone behaviors. The regulatory role of Ca^{2+} in growth cone extension has been suggested by the inverse correlation of neurite extension rate and the frequency of Ca^{2+} transients in growth cones, both *in vitro* and *in vivo* [64,65••]. Growth cone collapse is sometimes associated with a large increase in the cytosolic concentration of Ca^{2+} [66], whereas lowering extracellular Ca^{2+} levels may increase the rate of neurite extension [53••,65••]. The turning response of growth cones of cultured *Xenopus* spinal neurons induced by netrin-1, acetylcholine (ACh), BDNF and MAG, but not by NT-3 and Sema III, can be abolished by removing extracellular Ca^{2+} [13•,27••,53••,67]. Many signaling pathways can affect the level of cytosolic Ca^{2+} by inducing either Ca^{2+} influx through plasma membrane channels or Ca^{2+} release from internal stores [68]. For example, neural cell adhesion molecules, such as L1 and NCAM, can increase intracellular Ca^{2+} by opening Ca^{2+} channels [62]. Calcium release induced by inositol 1,4,5-trisphosphate (IP_3) in the growth cone appears to have a crucial role in controlling nerve growth [69•].

Calcium signaling can be transduced by calmodulin (CaM) and CaM-dependent kinases. Growth-cone turning induced by ACh is blocked by KN62, an inhibitor of CaM kinase [67]. Deviations of axons in growth, fasciculation and pathfinding have been observed in *Drosophila* embryos in which Ca^{2+}/CaM function was selectively disrupted [70]. The level of CaM in the growth cone may be regulated by GAP43, a protein associated with regenerating axons [71]. Disruption of the gene encoding GAP43 specifically affects growth cone steering at the optic chiasm in mice [71]. Another potential downstream target of Ca^{2+} is adenylyl

Figure 1



A summary of cyclic-nucleotide-dependent signaling pathways shared by two groups of guidance cues, derived from studies of cultured *Xenopus* spinal neurons [13•,27••,53••,84•]. The first pathway is Ca^{2+}-dependent and can be modulated by cAMP-dependent activity. Group I cues BDNF, NGF, netrin-1, ACh, and MAG appear to transduce their signals through this pathway. Although these cues have different receptors – presumably triggering different initial signals at the surface of the growth cone – they share a common downstream pathway, including Ca^{2+}, PI-3 kinase and PLC-γ pathways. The second pathway is independent of extracellular Ca^{2+} and PI-3 kinase, and can be modulated by cGMP-dependent activity. This pathway mediates the responses of the growth cones to Sema III and NT-3, group II cues. AChR, acetylcholine receptor.

cyclase [72]. Type I and type VIII adenylyl cyclases are stimulated by Ca^{2+} and CaM *in vivo* to produce cAMP, another important second messenger in the growth cone.

Cyclic nucleotides

Evidence is accumulating that cAMP and cGMP are key regulators for growth cone motility and axon guidance. *In vivo* loss of adenylyl cyclase I activity in mutant mice disrupts patterning of mouse somatosensory cortex [73••]. An optimal range of cAMP levels has been shown to be required for growth cone motility of cultured *Drosophila* neurons [74]. Growth cones of cultured spinal neurons show attractive turning in a gradient of a membrane permeable analogue of cAMP [75]. Depending on the level of cyclic nucleotides within the neuron, the response of the growth cone to many guidance cues can be either attractive/growth-promoting or repulsive/growth-inhibiting, with high levels favoring attraction and low levels favoring repulsion ([13•,27••,53••]; see below).

Cyclic AMP-dependent pathways can be modulated by many signals, including neurotransmitters, neuromodulators, adhesion molecules and Ca^{2+}. For example, activation of integrins can suppress intracellular cAMP levels [76], suggesting that different substrates may modulate the growth cone response to various guidance cues. Indeed, attraction of cultured retinal neuron growth cones by netrin-1 and BDNF is converted to repulsion when these neurons are grown on a laminin substrate (instead of polylysine) or in the presence of a specific peptide of the laminin B1 chain (V Hopker, M-m Poo, C Holt, unpublished observations). The effect of cAMP can be mediated by protein kinase A (PKA), whose substrates include IP_3 receptors [77] as well as cytoskeleton-associated proteins. For example, phosphorylation by PKA can switch off the activity of oncoprotein 18, a regulator of microtubule dynamics [78]. Mena, a profilin-binding protein required for axon guidance in mice, is also an *in vivo* substrate of PKA [23]. PKA-dependent phosphorylation of RhoA, a member of the small GTP-binding proteins involved in regulating cytoskeleton, leads to termination of RhoA signaling [79,80].

Cyclic GMP is produced by two different classes of guanylyl cyclases [81]. Soluble guanylyl cyclases are heterodimers that can be activated by the binding of gaseous molecules, such as nitric oxide and carbon monoxide. Receptor (particulate) guanylyl cyclases contain transmembrane domains and can be activated by extracellular ligands or by Ca^{2+} [81]. Nitric oxide and cGMP have been implicated in regulating the establishment of the central connections of developing retinal and olfactory axons [82,83]. Protein kinase G (PKG), a serine/threonine-specific protein kinase, is the major intracellular mediator of cGMP actions. Known PKG substrates include G_i and the type I IP_3 receptor [77].

Shared signaling mechanisms for two groups of guidance cues

Recent *in vitro* studies on the growth cone turning behaviors of *Xenopus* spinal neurons [13•,27••,53••,84•] have shown that all effective guidance cues for these neurons

can be classified into two groups, each sharing distinct cytoplasmic signaling pathways (see Figure 1).

In group I, which includes netrin-1, BDNF, NGF, ACh, and MAG, the turning responses are abolished by depletion of extracellular Ca^{2+}. Co-activation of PI-3 kinase and PLC-γ pathways are also required for the turning response [84•]. The level of cytosolic cAMP or the activity of 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, NGF, or ACh into repulsion, whereas activation of PKA converts repulsion induced by MAG into attraction. Consistent with this scheme, activation of PKA in NGF-stimulated PC12 cells prevents lysophosphatidic-acid-induced Ca^{2+}-dependent growth cone collapse and neurite retraction [85]. Elevation of endogenous cAMP levels also overcomes BDNF-induced growth cone collapse of cultured young *Xenopus* spinal neurons [55], and blocks the inhibition of axonal regeneration of cerebellar and sensory neurons by MAG and myelin [86].

In group II guidance cues, which includes Sema III and NT-3, the turning response is independent of extracellular Ca^{2+} or PI-3 kinase, and is regulated by cGMP or PKG [53••,84•]. Activation of PKG converts repulsive turning induced by Sema III into attraction, whereas inhibition of PKG converts NT-3-induced attraction into repulsion.

Consistent with the idea of two independent pathways shared by two groups of guidance cues, we [84•] found that the uniform presence of a high concentration of one guidance cue abolishes the turning response induced by a gradient of a second cue within the same group but not the other group.

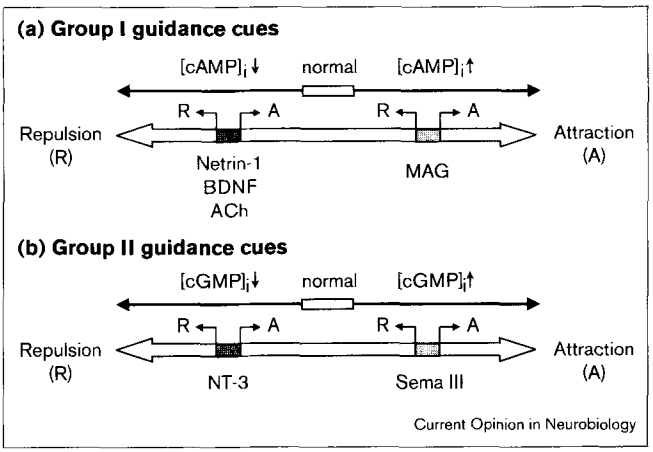
The response of a growth cone to a particular guidance cue tested in the above studies can be either attractive or repulsive, depending on the level of cyclic nucleotides. Thus, all these guidance cues can be defined as either attractants or repellents. The actual action of a guidance cue is mainly determined by the level of cyclic nucleotides within the neuron. Many extracellular ligands, including neuromodulators and adhesion molecules [75], can change the level of cyclic nucleotides, and are thus capable of modulating the growth cone response to a guidance cue when they are presented concurrently to a cell. For example, activation of metabotropic glutamate receptors by an agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid converts the turning response of *Xenopus* neurons in a gradient of BDNF from attraction into repulsion [53••]. Laminin, an ECM molecule, causes similar conversion of *Xenopus* retinal neurons in a netrin-1 or BDNF gradient (V Hopker, M-m Poo, C Holt, unpublished observations). Thus, during pathfinding, a growth cone not only receives guidance cues, but also acts as a coincidence detector to integrate all signals received from the environment to navigate towards its target.

In studies on *Xenopus* spinal neurons, the growth cone exhibits different ranges of cyclic nucleotide levels, referred to as the critical range, for conversion between an attractive and a repulsive response to each guidance cue (see Figure 2). When the basal level of cAMP (group I) or cGMP (group II) is above the critical range, a guidance cue causes attraction. Conversely, when the basal level is below the critical range, the guidance cue causes repulsion. Different critical ranges appear to exist for different guidance cues, although the precise values remain to be determined. To account for the cyclic-nucleotide-dependent conversion of growth cone responses in molecular terms, we propose a working molecular model involving cyclic-nucleotide-dependent phosphorylation of proteins that regulate polymerization and depolymerization of the cytoskeleton (Figure 3). Proteins of the Rho family are good candidates for the key components of this model, because they are known to modulate the cytoskeleton [87] and their activity is regulated by PKA [79,80].

Small GTPases of the Rho family

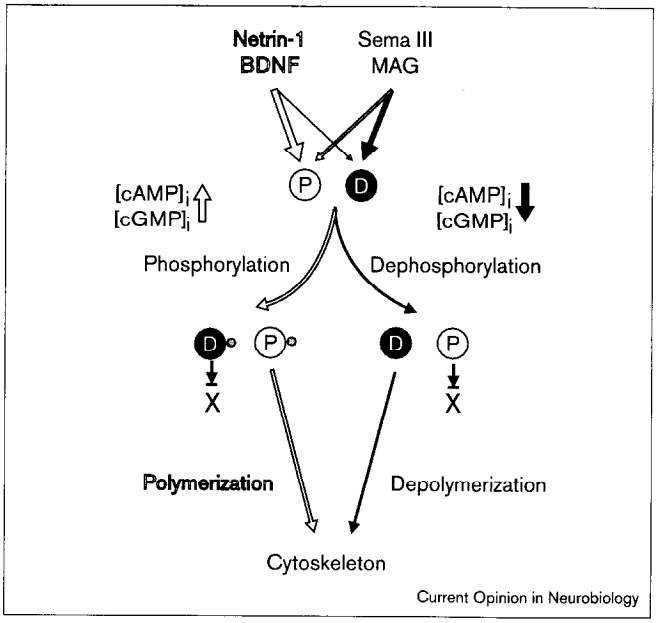
Members of the Rho family of small guanosine triphosphatases (GTPases, such as Rho, Rac, Cdc42) have recently emerged as key regulators of the actin cytoskeleton [87], the essential component for growth cone motility. These proteins modulate the actin cytoskeleton, resulting in differential effects on filopodia and lamellipodia. Whereas Rho regulates actin stress fiber formation and focal adhesion, Rac1 and Cdc42 regulate formation of lamellipodia

Figure 2



Critical range of cyclic nucleotides in setting growth-cone turning behaviors. For different guidance cues, the growth cone exhibits different ranges of cyclic nucleotide levels for conversion between attractive and repulsive responses. When the cytosolic level of **(a)** cAMP (group I) or **(b)** cGMP (group II) is above the critical range, a particular guidance cue causes attraction (A). When the level is below the range, repulsion (R) is induced. For factors that induce attraction under normal conditions (such as netrin-1 and BDNF), the critical range is low. The level of cytosolic cyclic nucleotides under normal conditions is above the range. Reduction of the cyclic nucleotide level converts attraction to repulsion. Conversely, factors that induce repulsion (such as MAG and Sema III) have a high critical range. $[cAMP]_i$, cytosolic concentration of cAMP; $[cGMP]_i$, cytosolic concentration of cGMP.

Figure 3



A working molecular model for the cyclic-nucleotide-dependent switch of growth-cone turning responses. The key elements of this model consist of a pair of components **D** and **P**, each with specific properties. First, activation of **P/D** leads both to polymerization/depolymerization of cytoskeletal structures and to growth cone extension/retraction, respectively. A gradient of activation of **P/D** across the growth cone results in preferential extension/retraction on one side, hence attractive/repulsive turning responses, respectively. Second, **D/P** can be activated by different diffusible factors to different degrees. Factors that trigger attraction/repulsion strongly, activate **P/D** and weakly activate **D/P**, respectively. Third, the function of **P/D** is gated by cyclic nucleotides (either cAMP or cGMP): phosphorylation of **P** and **D** by PKA or PKG favors the function of **P** but not that of **D**. Conversely, dephosphorylation of **P** and **D** (due to basal phosphatase activities at low cyclic nucleotide levels) favors function of **D** but not that of **P**. In this model, we assume that the motility of the growth cone is a balance between polymerization and depolymerization of the cytoskeleton. A net polymerization/depolymerization leads to extension/retraction of the growth cone. In the presence of a gradient of guidance cues, attractive/repulsive turning is attributable to a net polymerization/depolymerization on the side of the growth cone facing the gradient, respectively. Thus, the direction of the growth cone is determined by a gradient of activity of **P** and **D** triggered by a gradient of the guidance cue. In this model, **P** and **D** can be two pathways or simply two molecules, or even part of a single molecule, provided that the activity of this molecule produces opposite effects on cytoskeleton structures when phosphorylated or dephosphorylated. It is possible that different signaling cascades triggered by different factors converge on **P/D**, and **P/D** acts downstream in the cascade closer to cytoskeletal structures.

and filopodia, respectively [87]. Expression of constitutive or dominant-negative forms of Rac1 and Cdc42 cause defects in axon guidance and cell migration in *C. elegans*, *Drosophila*, and mouse [87]. It has been speculated that, in the growth cone, Cdc42 and Rac are under the control of attractive guidance cues and Rho is under the control of repulsive guidance cues. Many regulators and downstream effectors of this family of proteins have been identified [87], but the role of most of them in axonal guidance is unknown. Dock, an adapter protein essential for photoreceptor (R cell) guidance and target recognition in the *Drosophila* visual system, has been shown to genetically interact with Cdc42, suggesting functional roles of Dock and Cdc42 in the same signaling pathway [88].

Conclusions

Significant progress has been made in understanding the molecular mechanisms of axon guidance at all levels, including identification of guidance cues, their receptors and cytoplasmic signal transduction pathways. More diffusible guidance cues and their receptors are likely to be identified in coming years. Intracellular cascades for all these diverse ligand–receptor systems, while unclear at present, are likely to be transduction mechanisms commonly used by many other cellular signaling processes. Two cyclic–nucleotide-dependent pathways have emerged as the shared transduction mechanism for two separate groups of guidance cues. Future studies will help to delineate the molecules that link these pathways upstream to receptors for guidance cues and downstream to effectors responsible for cytoskeletal rearrangements underlying the turning responses.

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