Semaphorin Regulation of Cellular Morphology

Tracy S. Tran, Alex L. Kolodkin, and Rajnish Bharadwaj

The Solomon H. Snyder Department of Neuroscience, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; email: kolodkin@jhmi.edu

Key Words
axon guidance, plexin signaling, cell migration, cardiovascular development

Abstract
Semaphorin proteins, although initially characterized as repulsive neuronal guidance cues, are now appreciated as major contributors to morphogenesis and homeostasis for a wide range of tissue types. Semaphorin-mediated long- and short-range repulsive, and attractive, guidance has profound influences on cellular morphology. The diversity of semaphorin receptor complexes utilized by various semaphorin ligands, the ability of semaphorins themselves to serve as receptors, and the myriad of intracellular signaling components that comprise semaphorin signaling cascades all contribute to cell-type-specific responses to semaphorins. Analysis of the molecular and cellular mechanisms underlying semaphorin function in neural and vascular systems provides insight into principles governing how this large protein family contributes to organogenesis, function, and disease.
INTRODUCTION

The establishment of elaborate and precise patterns of neural connectivity during development depends critically upon a large variety of attractive and repulsive guidance cues. These cues function to control the direction of axonal and dendritic growth and the branching of neuronal processes, and they play key roles in defining the mature pattern of synaptic connectivity. Guidance cues also direct neuronal migration events essential for allowing neurons to assume positions appropriate to the circuits they will ultimately construct (Dickson 2002, Tessier-Lavigne & Goodman 1996). Patterning of neuronal connections occurs with astonishing precision during development, largely through the action of specialized structures at the leading edge of neuronal processes called growth cones. As they migrate to their target area and contact their postsynaptic partners, neuronal growth cones perceive and interpret attractive and repulsive guidance cues. This process relies heavily on a complex network of intercellular signaling pathways, and the coordinated activation of these pathways is dependent upon the tightly regulated spatial and temporal distribution of guidance cue ligands and their cognate receptors. Growth cone steering also depends upon the presence of the intracellular molecular components essential for signaling events capable of translating extracellular cues into discrete changes in cellular morphology. In many ways, the cytoskeletal events involved in steering neuronal growth cones are quite similar to those operating at the leading edge of migrating cells (Ridley et al. 2003), and indeed several axon guidance cues can regulate both neuronal migration and process morphology. Therefore, the extensive work on neuronal guidance cue function provides insight into a wide range of cellular events essential for organogenesis.

Semaphorins constitute one of the largest protein families of phylogenetically conserved guidance cues (Semaphorin Nomenclature Committee 1999), and they have been extensively studied in Caenorhabditis elegans, Drosophila, zebrafish, rodents, and humans. Secreted and membrane-bound semaphorins participate in diverse biological processes, including central and peripheral nervous system (CNS and PNS, respectively) development and regeneration, cardiovascular development, and immune system function (Kikutani & Kumanogoh 2003, Kruger et al. 2005, Pasterkamp & Verhaagen 2006, Yazdani & Terman 2006). Although initially identified as axonal repellents, semaphorins, like most guidance cue families, include both attractants and repellents. Moreover, many
Semaphorin proteins are bifunctional, and both intrinsic and extrinsic modulatory factors serve to facilitate repulsive or attractive functions.

Here, we address how semaphorins direct a variety of patterning events that require regulation of cellular morphology. Our goal is to present examples of the diversity inherent in semaphorin-mediated guidance, to highlight recent advances in our understanding of semaphorin-mediated guidance, and to discuss cellular and molecular principles underlying the action of these guidance cues. In most cases these concepts can be applied to general guidance cue function. Following an overview of semaphorin ligand–holoreceptor complex interactions, we review select neuronal guidance events that rely on semaphorin signaling, including those affecting pathfinding, process morphology, and cell migration. We also discuss how semaphorin signaling regulates nonneuronal cell morphology in the context of cardiovascular development. Finally, we consider intracellular signaling events downstream of semaphorin receptor engagement that provide additional insight into the molecular mechanisms by which growth cones respond to semaphorins.

**SEMAPHORINS AND THEIR RECEPTORS**

Semaphorin proteins are defined by a ∼500-amino-acid conserved semaphorin (sema) domain common to all family members and, with the exception of certain viral semaphorins, a short plexin-semaphorin-integrin (PSI) domain (Gherardi et al. 2004, Semaphorin Nomenclature Committee 1999). Semaphorins are further distinguished by the presence of additional class-specific domains and, on the basis of domain organization, are grouped into eight subfamily classes (Figure 1). Semaphorins in classes 1 and 2, and one in class 5 (Sema5c), are found in invertebrates. Classes 2, 3, and V semaphorins are secreted, whereas semaphorins in all other classes are membrane tethered. The diversity inherent in semaphorin protein structure provides the basis for these cues to serve short- or long-range guidance functions, to interact with a complex array of distinct receptor (or holoreceptor) complexes, and to act themselves as receptors. Although semaphorins were initially best characterized as neuronal repellents (Raper 2000), we know now that semaphorins can repel or attract a wide range of neuronal and nonneuronal cells.

By far the most prominent semaphorin receptors are the plexin proteins. Plexins are large, phylogenetically conserved transmembrane proteins that in both invertebrates and vertebrates transduce semaphorin guidance signals (Kruger et al. 2005, Tamagnone & Comoglio 2000). Plexins can be divided into four classes (A–D), contain a divergent extracellular semaphorin domain and a conserved cytoplasmic domain unique to plexins, and include nine members in vertebrates (Figure 1). Fujisawa and colleagues initially observed that a plexin protein in *Xenopus* was expressed selectively on subsets of accessory and main olfactory sensory neurons, leading to the speculation that plexins organize axonal trajectories (Satoda et al. 1995). The first appreciation that plexins play key roles in mediating semaphorin signaling came from the observation that Plexin C1 (PlexC1) on B cells binds to the viral semaphorin A39R, resulting in monocyte activation (Comeau et al. 1998). Subsequently, Plexin A (PlexA) in *Drosophila* was shown to bind class 1 semaphorins and mediate Sema-1a-dependent motor and CNS axon guidance in vivo (Winberg et al. 1998a). Extensive analyses demonstrate that vertebrate plexins signal both secreted and transmembrane semaphorin-mediated repulsive neuronal guidance and that the plexin cytoplasmic domain is critical for these signaling events (Cheng et al. 2001, Suto et al. 2005, Swiercz et al. 2002, Takahashi et al. 1999, Tamagnone et al. 1999, Yaron et al. 2005, Yoshida et al. 2006). Therefore, to understand how semaphorins influence cellular morphology, it is crucial to define the signaling pathways...
downstream of plexin receptor activation, to understand how plexin signaling can be modulated, and to place the plexin signaling cascade in the context of the network of signal transduction pathways known to influence cellular process outgrowth and migration.

Although plexins serve as receptors for all classes of semaphorins, the repertoire of

Figure 1

Semaphorins and their receptors. Semaphorins (semas) include transmembrane, secreted, and glycosylphosphatidylinositol (GPI)-linked proteins, most of which bind to plexin receptors. Class 1 and 2 and Sema5c semas are invertebrate, and class 1 and 2 semas utilize PlexinA (PlexA) and PlexinB (PlexB) receptors. The coreceptor D-OTK functions with PlexA. Semas in classes 3–7 are found in vertebrates. Class 3 and 6 semas utilize PlexA receptors; however, most Sema3s require an obligate neuropilin (Npn) coreceptor. Semas interact directly with PlexAs. Other coreceptors that can function with PlexAs are L1CAM (L1 cell adhesion molecule), vascular endothelial growth factor receptor 2 (VEGFR2), and Offtrack (OTK). Class 4 and 5 semas associate with PlexB1–3, and the receptor tyrosine kinases (RTKs) Met and ErbB2 can function as a coreceptor with PlexBs for certain class 4 sema functions. Class 4 semas directly bind to CD72 or Tim-2 in the immune system. Sema7A and a viral sema function together with PlexC1, and Sema7A also utilizes neuronal integrin receptors. Domains: C1/C2, intracellular 1 and 2; cleavage site, convertase cleavage site; Cub, complement binding; FIII, fibronectin type III; FV/VIII, coagulation factor; Ig-like, immunoglobulin-like; IPT, Ig-like, plexins, transcription factor domains; MAM, “Meprin, A5, Mu”; PDZ, PDZ binding site; PSI, plexin-semaphorin-integrin domain; SemaP, sema domain of plexins; SemaS, sema domain of semaphorins; TR, thrombospondin; TK, tyrosine kinase.
Semaphorin-plexin signaling is greatly expanded by numerous coreceptors that serve to define semaphorin holoreceptor complexes (Figure 1). For example, most vertebrate class 3 secreted semaphorins do not bind directly to A-class plexins but instead bind to the related transmembrane proteins Neuropilin (Npn)-1 or -2 to assemble and activate a Npn-plexin holoreceptor complex (Fujisawa 2002, Huber et al. 2003, Raper 2000). In addition, class 3 semaphorin signaling complexes can require the immunoglobulin (Ig) superfamily members L1CAM or NrCAM (where CAM denotes cell adhesion molecule) for specific repulsive or attractive neuronal guidance events (Castellani et al. 2000, Julien et al. 2005). In both invertebrates and vertebrates, the receptor tyrosine kinase (RTK)-like transmembrane protein offtrack (OTK) appears to form a complex with PlexA receptors and is required for neuronal and nonneuronal transmembrane semaphorin-mediated guidance (Toyofuku et al. 2005, Winberg et al. 2001). Interestingly, the scatter factor/hepatocyte growth factor receptor Met, a classical RTK that, unlike OTK, contains a catalytically active kinase domain, can form a holoreceptor complex with PlexinB1 (PlexB1) in nonneuronal cells, facilitating class 4 transmembrane semaphorin activation of Met and stimulating invasive growth in epithelial cells (Giordano et al. 2002). ErbB2, another classical RTK, also can form a functional holoreceptor complex with PlexB1 and plays a key role in Sema4D-mediated neuronal growth cone collapse (Swiercz et al. 2004). PlexA receptors also have the capacity to employ RTKs as coreceptors, and PlexA1-VEGFR2 holoreceptor complexes are required for Sema6D-mediated signaling in cardiac development (Toyofuku et al. 2004a). Although these coreceptors define plexin-containing semaphorin holoreceptor complexes with unique signaling capacities, plexin-independent semaphorin receptor signaling provides further diversity for semaphorin function. Class 4 semaphorins signal through two immune system receptors, CD72 and Tim-2 (Kumanogoh & Kikutani 2003). And in certain classes of neurons, the glycosylphosphatidylinositol (GPI)-anchored Sema7A promotes outgrowth through the activation of integrin receptors (Pasterkamp et al. 2003). Therefore, we can expect that cell-type-specific expression of distinct semaphorin receptors and coreceptors will play key roles in dictating how these guidance cues exert selective effects on cellular morphology.

**SEMAPHORIN-MEDIATED REPULSIVE REGULATION OF PROCESS MORPHOLOGY**

**Regulation of Fasciculation, Pathfinding, and Branching**

One of the best-defined semaphorin functions is that of an extrinsic repellent: maintaining nerve bundling by preventing axon defasciculation, defining permissive pathways for process extension, and sculpting arborization patterns by constraining axon extension and branching. The pattern of peripheral neuronal projections that evolves during vertebrate neural development depends critically upon surround repulsion of secreted semaphorins. For example, Sema3A, originally called collapsin-1 and the first vertebrate semaphorin identified, is a potent sensory and sympathetic neuronal growth cone–collapsing factor (Luo et al. 1993). Furthermore, Sema3A has the capacity to inhibit axon branching in cortical neurons (Dent et al. 2004). During vertebrate embryonic development, Sema3A is expressed in regions surrounding peripheral trigeminal nerve projections to the eye and branchial arches, in developing somites delineating spinal sensory and motor projections, and in the developing limb bud. In Sema3A mutant mice, select cranial nerves, including the ophthalmic nerve and to a lesser extent the maxillary and mandibular branches of the trigeminal nerve, exhibit extensive defasciculation and branching defects during embryogenesis (Taniguchi
Sympathetic ganglia (SG): components of the sympathetic nervous system whose cell bodies are found in connected ganglia located along either side of the spinal cord.


et al. 1997) (Figure 2a). Moreover, spinal nerve projections are no longer restricted to their normal pathways in the anterior somite or to proximal portions of the developing limb, and sympathetic neuronal projections are also disorganized. The related semaphorin Sema3F plays a similar role for an overlapping, but distinct, set of cranial nerves consistent with its unique expression pattern (Chen et al. 2000, Giger et al. 2000, Sahay et al. 2003).

Analysis of PlexA3 and PlexA4 null mutant mice demonstrates that plexins indeed serve as functional neuronal vertebrate class 3 secreted semaphorin receptors in vivo and that distinct functions of different semaphorin guidance cues are linked to selective receptor engagement (Cheng et al. 2001, Suto et al. 2005, Yaron et al. 2005). For example, the sympathetic ganglion (SG) neurons derived from PlexA4 mutant embryos show a dramatic decrement in the degree to which they are repelled by Sema3A; however, their repulsion by Sema3F remains normal. In contrast, SG neurons taken from PlexA3 mutant embryos are no longer repelled by Sema3F but are still repelled by Sema3A. In vivo, PlexA4 mutants exhibit profound disruption of peripheral spinal sensory and motor projections, similar to but less severe than what is observed in Sema3A mutants. In contrast, PlexA3 and Sema3F mutant mice are indeed repelled by Sema3A, and this is supported by the observation that SG axons from PlexA3/PlexA4 double-mutant mice are completely unresponsive to Sema3A (Yaron et al. 2005). The greater severity of cranial and spinal nerve defasciculation and arborization defects observed in PlexA3/PlexA4 double-mutant mice, compared with either single mutant alone, also suggests that PlexA3 is required for some aspects of Sema3A-mediated repulsion in vivo. Additionally, Sema3F, but not Sema3A, mutant mice exhibit severe trochlear nerve pathfinding defects (Sahay et al. 2005), yet this defect is phenocopied only in PlexA3/PlexA4 double mutants and not in either PlexA3 or PlexA4 single mutants (Yaron et al. 2005).

This suggests that both PlexA3 and PlexA4 are also required for Sema3F repulsion in certain contexts. Therefore, although plexins signal secreted semaphorin repulsion, the interactions between these secreted repellents and other observations support the idea that Sema3A and Sema3F signal repulsion primarily through the action of PlexA4 and PlexA3, respectively.

Interestingly, although mutant PlexA3 SG neurons are indeed repelled by Sema3A, this response is somewhat weaker than that of wild-type SG neurons. Furthermore, PlexA4 mutant SG neurons retain a greatly reduced capacity to be repelled by Sema3A. These observations suggest that both PlexA3 and PlexA4 can mediate Sema3A repulsion, and this is supported by the observation that SG axons from PlexA3/PlexA4 double-mutant mice, compared with either single mutant alone, also suggests that PlexA3 is required for some aspects of Sema3A-mediated repulsion in vivo.

Semaphorins regulate process fasciculation and branching through inhibition. (a) Expression of Sema3A (gray) creates boundaries for the pathfinding and arborization of cranial and spinal nerves in vertebrate embryos. In Sema3A mutant mouse embryos, these nerves are defasciculated and show exuberant arborization into regions that express Sema3A. (b) Sema3E (yellow) is expressed in somites and defines the boundaries of intersomitic vessel extension and branching. Intersomitic vasculature in Sema3E mutants exhibits excessive branching and extension over normal somitic boundaries. (c) Medial lateral motor column (LMCm) neurons, which express the Sema3F coreceptor Npn-2 and the Sema3A coreceptor Npn-1, extend into the ventral mesenchyme of the developing limb bud. However, lateral LMC (LMCl) neurons, which express Npn-1 but not Npn-2, extend into the dorsal limb bud (i). Sema3A is expressed in regions surrounding the motor neuron trajectories, and Sema3A and Npn-1 mutants show aberrant defasciculation of motor nerves. This results in incorrect extension of some LMCm and LMCl axons into the dorsal and ventral limb bud, respectively (ii). Sema3F expressed in the dorsal limb prevents LMCm neurons from invading the dorsal limb bud, and in Sema3F or Npn-2 mutants LMCm axons aberrantly extend into the dorsal limb; however, LMCl neurons still project normally (iii).
a) Wild type vs. Sema3A−/−

b) Wild type vs. Sema3E−/−

c) i) Wild type
   ii) Sema3A−/− or Npn-1−/−
   iii) Sema3F−/− or Npn-2−/−

Legend:
- Sema3A
- Sema3E
- Npn-1 (LMCl)
- Npn-2 (LMCm)
- Midline
and individual plexin receptors alone do not completely account for guidance events mediated by several of these repulsive cues. Plexin receptor–specific interactions with cytosolic signaling cofactors, or even heteromultimeric plexin receptor associations, may in part underlie distinct and shared plexin signaling in response to semaphorin ligands. However, most secreted semaphorins, including Sema3A and Sema3F, do not bind directly to PlexA receptors. It therefore falls to coreceptors to complete functioning holoreceptor complexes that facilitate individual neuron-specific responses to many semaphorins.

The transmembrane proteins Npn-1 and Npn-2 constitute a small family of obligate coreceptors for several secreted semaphorins (Figure 1) (Chen et al. 1997, Fujisawa 2002, He & Tessier-Lavigne 1997, Kolodkin et al. 1997). Npn-1 binds with high affinity to Sema3A, whereas Sema3F binds to Npn-2. The short neuropilin cytoplasmic domain is dispensable for repulsive signaling, and binding of a secreted semaphorin to a neuropilin–plexin holoreceptor complex serves to activate the plexin cytoplasmic domain. This is achieved through the abrogation of plexin receptor autoinhibition resulting from intramolecular interactions between portions of the plexin receptor ectodomain (Takahashi & Strittmatter 2001). Npn-1 and Sema3A mutant mice exhibit very similar neuronal guidance phenotypes (Gu et al. 2003, Kitsukawa et al. 1997, Taniguchi et al. 1997), as do Npn-2 and Sema3F mutant mice (Chen et al. 2000, Giger et al. 2000, Sahay et al. 2005). Indeed, Npn mutant phenotypes are much closer to those observed in mice harboring mutations in either Sema3A or Sema3F mutants, and Npn-1 and Npn-2 are expressed selectively in those populations of neurons affected in either Npn-1 or Npn-2 mutants. Therefore, the ability of certain secreted semaphorins to selectively guide unique neuronal populations through surround repulsion depends critically upon the definition of holoreceptor signaling specificity by neuropilin coreceptors.

Secreted semaphorins also restrain branching and process outgrowth in nonneuronal cell types. One excellent example of this is observed in developing somitic vasculature. In zebrafish and mice, secreted semaphorins are expressed in regions avoided by sprouting and growing intersomitic vessels (Gitler et al. 2004, Gu et al. 2005, Torres-Vazquez et al. 2004). In mouse embryos, the secreted semaphorin Sema3E is expressed adjacent to somite boundaries and the intersomitic vasculature in the caudal region of each somite. In Sema3E mutant embryos, the iterative pattern of the intersomitic vasculature is disorganized, and vessels extend ectopically across somite boundaries (Figure 2b) (Gu et al. 2005). Although this defect is reminiscent of peripheral spinal nerve phenotypes observed in Sema3A mutant mouse embryos, and although the somitic distributions of Sema3E and Sema3A are also similar, neither Npn-1 nor Npn-2 is required for the normal generation of intersomitic vascular patterning (Gu et al. 2005). Furthermore, intersomitic vascular defects observed in Sema3A mutant mouse embryos result in a reduction in, not an expansion of, vascular branching and extension (Serini et al. 2003). Therefore, it is not surprising that Sema3E repulsive effects on endothelial cells require signaling through a distinct semaphorin receptor. In zebrafish and mouse, mutations in the gene encoding the divergent plexin receptor PlexinD1 (PlexD1) (Figure 2b) phenocopy the vascular defects observed in Sema3E mutants (Gitler et al. 2004, Gu et al. 2005, Torres-Vazquez 2005).
et al. 2004). It will be interesting to learn whether the use of a different plexin receptor for secreted semaphorin–mediated surround repulsion in endothelial cells defines unique intracellular signaling events required for the generation of vascular networks.

The wealth of ligand-receptor interactions capable of transducing semaphorin signaling events provides an opportunity for differential semaphorin receptor expression in subsets of related cell types, and similarly diverse semaphorin expression patterns in intermediate and final target regions, to produce complex patterns of cellular morphology and projections. For example, the elaboration of distinct spinal-motor-neuron projection patterns in the developing vertebrate limb is dependent in part on selective repulsive interactions between different secreted semaphorins and groups of motor neurons that differentially express receptors for these repellents. Secreted semaphorins can repel spinal motor neurons (Varela-Echavarria et al. 1997), and populations of motor neurons in the developing vertebrate spinal cord express various semaphorin receptors, including neuropilin coreceptors (Cohen et al. 2005, Feldner et al. 2005, Huber et al. 2005). At limb levels in the developing vertebrate spinal cord, motor neurons are initially organized into medial and lateral motor columns (MMC and LMC, respectively), and from the MMC and LMC motor neuron projections extend to their intermediate and final targets (Jessell 2000). The LMC is further divided into lateral (LMCl) and medial (LMCm) divisions; motor neurons that settle in the LMCl project their axons to dorsal limb muscles, and LMCm neurons project to the ventral limb (Figure 2c). Upon exiting the spinal cord, motor neuron axons extend within stereotypic nerve trajectories to the dorsal-ventral choice point within the developing limb, and there they establish trajectories to their appropriate target muscles. Interestingly, when motor axons exit the spinal cord, LMCl and LMCm neurons both express Npn-1; however, only LMCm neurons express Npn-2. The Npn-1 ligand Sema3A is expressed throughout the developing limb bud as motor axons initially enter the limb and later surrounds motor axon trajectories in the proximal portion of the limb. In the absence of either Npn-1 or Sema3A, motor axons enter the limb precociously, motor axon projections are disorganized and defasciculated in the proximal limb, and both LMCl and LMCm motor neurons misproject to the ventral limb and dorsal limb, respectively (Figure 2c). In contrast, the Npn-2 ligand Sema3F is expressed in the dorsal portion of the developing limb and, unlike Sema3A, does not surround motor axon projections in the limb. In the Sema3F or Npn-2 mutant mice, Npn-2-expressing LMCm neurons misproject to the dorsal limb, but LMCi neurons project normally to the dorsal limb (Figure 2c) (Huber et al. 2005). Therefore, differential expression of neuropilin coreceptors in motor neurons, and of their cognate ligands in the developing limb bud, controls distinct steps of motor axon growth and guidance crucial for correct pathfinding and target innervation.

In contrast to these examples of semaphorins acting to restrict process extension and branch formation through the provision of repulsive barriers distinct from the processes themselves, semaphorin signaling also plays a key role in regulating axon fasciculation through repulsive axon-axon interactions within nerve bundles. These repulsive interactions among axons likely serve to counter axon-axon adhesion, allowing subsets of axons to separate from nerve bundles and extend along distinct pathways. This is best observed in the developing Drosophila embryo, in which analysis of motor axon trajectories allows for a detailed assessment of guidance cue effects on successive, stereotypic defasciculation events (Landgraf et al. 1997, Vactor et al. 1993). Motor axons in Drosophila exit the CNS in nerve bundles, from which axons successively defasciculate as they extend toward their peripheral target muscles. The transmembrane semaphorin Sema-1a and its receptor PlexA are expressed
on most, if not all, Drosophila motor axons, and in the absence of either Sema-1a or PlexA motor neurons remain hyperfasciculated, often failing to innervate their appropriate targets. If Sema-1a signaling is hyperactivated, through overexpression of either Sema-1a, PlexA, or downstream signaling components required for semaphorin repulsive axon-axon interactions, then motor axons defasciculate prematurely, exhibiting profound disruption of the normal pattern of motor nerves (Ayoob et al. 2004, Terman & Kolodkin 2004, Terman et al. 2002, Winberg et al. 1998a, Yu et al. 1998). Coupled with observations showing that ectopically expressed Sema-1a inhibits motor axon extension in vivo and that adhesion molecules serve to counter the effects of Sema-1a on motor axon fasciculation (Winberg et al. 1998b, Yu et al. 1998, Yu et al. 2000), these results demonstrate that Sema-1a signaling provides a level of repulsion among axons extending within the same nerve bundle that is crucial for generating appropriate patterns of motor nerves during the establishment of neuromuscular connectivity. Given the prevalence of transmembrane semaphorin expression in vertebrates, it will be interesting to determine how semaphorin signaling among neurons themselves directs complex defasciculation events. In addition to membrane-associated semaphorins, secreted semaphorins may also have cell-autonomous functions. Secreted semaphorins are themselves expressed selectively in multiple neuronal populations (for example, Cohen et al. 2005). Thus, the regulation of axon fasciculation and branching by semaphorins expressed on these processes is likely a general feature of semaphorin-mediated regulation of process bundling and morphology.

**Semaphorin Regulation of Axon Targeting**

Recent observations establish important roles—in addition to directing and regulating axon pathfinding, branching, and fascication—for semaphorin signaling in several neural systems to selectively direct subsets of related afferent projections to their appropriate CNS targets. For example, the segregation of proprioceptive and cutaneous sensory neurons to ventral and dorsal spinal cord target regions, respectively, provides a remarkable example of dynamic temporal and spatial regulation of targeting by distinct subpopulations of related neurons. Proprioceptive sensory neurons extend into the dorsal spinal cord, avoid the superficial dorsal horn (where cutaneous afferent fibers will ultimately project), and then extend ventrally to their motor neuron targets. Proprioceptive, but not cutaneous, sensory neurons express the PlexA1 receptor, and PlexA1 transmembrane semaphorin ligands Sema6C and 6D are found precisely in those regions of the dorsal horn devoid of proprioceptive axon trajectories (Yoshida et al. 2006). In PlexA1 mutant embryos, proprioceptive neurons still project to their ventral targets; however, the axon shafts of these axons subsequently invade the superficial dorsal horn, suggesting that Sema6C/6D-PlexA1 signaling regulates axon shaft positioning. Interestingly, the organization of cutaneous afferent terminations in the dorsal horn of PlexA1 mutants is also disrupted, and these defects are associated with displaced oligodendrocytes along the ventrally shifted proprioceptive axon shafts (Yoshida et al. 2006). Therefore, class 6 semaphorin–PlexA signaling plays a key role in regulating sensory axon segregation, allowing for correct patterning of sensory afferents within the developing spinal cord.

The topographic projections of retinal ganglion cells (RGCs) to the optic tectum involve targeting events for which recent results also suggest that semaphorin signaling plays a key role. Extensive work shows that ephrins and their Eph receptors direct retinal axons extending from neurons along the temporal-nasal retinal axis to their targets along the anterior-posterior axis of the tectum (McLaughlin & O’Leary 2005). Although ephrins also participate in establishing
veloping olfactory bulb, and effects include not only projections of ORNs targeting the main olfactory bulb (OB) (Cloutier et al. 2002, 2004; Walz et al. 2002). Recent results show that odorant receptor–derived cAMP directs main ORN targeting along the anterior-posterior axis of the olfactory bulb (OB) (Imai et al. 2006). Intriguingly, cAMP-mediated differential expression of axon guidance molecules, including Npn-1, results from olfactory receptor signaling during development. In addition to regulating guidance cue receptor expression, odorant receptor–mediated modulation of cAMP levels within ORNs may result in differential sensitivity of guidance receptors to OB guidance cues (Song et al. 1998). Taken together, all these results show that secreted semaphorins play a key role in directing distinct classes of mouse olfactory sensory neurons to contact glomeruli in appropriate target regions.

Very recent results show that semaphorin signaling plays a crucial role in assembling the Drosophila olfactory system as well (Komiyama et al. 2007, Latterman et al. 2007, Sweeney et al. 2007). The transmembrane semaphorin Sema-1a is expressed on Drosophila ORNs, and Sema-1a–PlexA signaling among ORNs functions nonautonomously to regulate correct targeting of these sensory neurons to appropriate glomeruli in the antennal lobe. Sema-1a–PlexA signaling also plays a key role in regulating entry of ORN subclasses into the antennal lobe (Latterman et al. 2007, Sweeney et al. 2007). These results suggest that Sema-1a-mediated repulsive interactions allow early-arriving ORN axons to constrain targeting of ORN axons that arrive in the antennal lobe at later times during the assembly of stereotypic ORN connectivity patterns. Remarkably, at an earlier stage of olfactory
Infrapyramidal bundle (IPB): a tract in the hippocampus composed of axons that extend from dentate granule cells in the dentate gyrus to basal dendrites of CA3 pyramidal neurons, projecting below the CA3 pyramidal layer system development. Sema-1a is also required cell-autonomously to direct the targeting of olfactory projection neuron dendrites to their correct location along the dorsolateral-ventromedial axis of the antennal lobe (Komiyama et al. 2007). In addition, Sema-1a also acts as a receptor to direct projection neuron axons to their correct targeting in higher olfactory centers in the lateral horn and mushroom body of the *Drosophila* brain. Therefore, Sema-1a plays distinct and critical roles in the orchestration of the ordered assembly of pre- and postsynaptic components of developing olfactory circuitry, both as a receptor for an as-yet-identified ligand to direct projection neuron targeting and as a PlexA ligand to direct ORN neuron targeting. It will be interesting to determine the degree to which the molecular mechanisms governing rodent and insect olfactory system connectivity are conserved.

**Semaphorin-Mediated Axonal Pruning**

Secreted semaphorins also direct the pruning of exuberant axon projections in the developing hippocampus, sculpting immature trajectories to establish their adult connectivity patterns. In *Plex-A3* and *Npn-2* mutant mice, infrapyramidal bundle (IPB) axons in the hippocampus, which originate in the dentate gyrus, remain overextended within the CA3 region into adulthood (Bagri et al. 2003, Chen et al. 2000, Cheng et al. 2001, Giger et al. 2000). *Sema3F* mRNA is expressed along the IPB at relatively high levels during late postnatal development, suggesting that it regulates the onset of IPB axonal pruning through the activation of Npn-2–PlexA3 receptor complexes. Consistent with this hypothesis, *Sema3F* in vitro stimulates the retraction of hippocampal axons (Bagri et al. 2003), and *Sema3F* mutant mice, similar to mice harboring mutations in its receptors, *Npn-2* and *PlexA3*, display IPB defects, including aberrant extension of IPB axons into the stratum oriens of the CA3 that persists into adulthood (Sahay et al. 2003). In addition to the IPB pruning defects, adult *PlexA3* mutant mice display aberrant projections from CA1 to the medial septum that are indicative of a failure to prune hippocampo-septal projections (Bagri et al. 2003). These pruning defects, however, appear to be mediated by Sema3A, not Sema3F, and are not observed in *Npn-2* mutants. Therefore, class 3 secreted semaphorins are capable of selectively activating a program of axonal pruning, but it remains to be determined how PlexA3 signaling prunes existing axonal projections. Plexin signaling may directly lead to the destabilization of synaptic complexes, which in turn results in axon collateral pruning. Both *Npn-2* and *PlexA3* mutants contain more mossy fiber synaptic complexes with CA3 dendritic spines than are observed in wild-type mice, and these contacts are significantly larger than normal (Liu et al. 2005). Plexin-mediated hippocampal mossy fiber and CA1 pyramidal neuron pruning appears to occur through axon retraction rather than classic wallerian degeneration or axosome shedding (Bagri et al. 2003, Liu et al. 2005). Microtubules and neurofilaments in hippocampal axons undergoing pruning appear intact, suggesting that fast and slow retrograde transport mechanisms may support Sema3F-mediated hippocampal axon retraction. It remains an open question whether secreted semaphorin-mediated axon pruning and repulsive axon guidance events utilize similar or distinct signaling mechanisms.

**BIFUNCTIONAL SEMAPHORIN SIGNALING**

A feature common to most guidance cues is their ability to function as an attractant or a repellent. The factors influencing this choice include intrinsic signaling events, extrinsic components, and guidance cue holoreceptor composition. Secreted class 3 semaphorins, although initially characterized as repellents, were rapidly shown to be bifunctional on the basis of the ability of certain semaphorins to function as attractants for cortical and
olfactory neurons in vitro (Bagnard et al. 1998, de Castro et al. 1999). Intriguingly, Sem3A repulsive steering of individual growth cones in culture can be switched to attraction by the elevation of intracellular cGMP signaling (Song et al. 1998), and in cortical slice overlay assays elevated cGMP signaling in cortical neuron dendrites promotes their attraction to Sem3A (Polleux et al. 2000). These and other observations demonstrate that intrinsic signaling has the capacity to influence the sign of neuronal growth cone responses to semaphorins. Recent work shows that in zebrafish and mouse the formation of the anterior commissure (AC), a major tract in the developing forebrain, requires secreted semaphorin attraction, and in the mouse both attraction and repulsion are necessary. Distinct secreted semaphorin holoreceptor composition plays a key role in determining whether secreted semaphorins are attractants or repellents for AC axons, and different neuropilin proteins or Ig superfamily CAMs are essential for these choices.

Zebrafish telencephalic neurons that form the AC require the secreted semaphorin Sem3D, which is expressed in the dorsolateral telencephalon, for guidance across the CNS midline (Wolman et al. 2004). Unlike mammals, zebrafish have four Npns, and Npn-1a and Npn-2b are both required for the generation of normal Sem3D-dependent AC projections across the CNS midline. Sem3D loss-of-function experiments show that this semaphorin is required for AC formation, and gain-of-function experiments show that Sem3D is an attractant for AC axons. In a different axonal trajectory, Sem3D requires only Npn-1a to repel axons from the medial longitudinal fasciculus, preventing them from contacting the midbrain and facilitating their caudal projection toward the hindbrain and spinal cord. Therefore, neuropilin contributions to Sem3D holoreceptor complexes presumably define Sem3D repulsive and attractive functions.

In the mouse, the AC includes both an anterior (ACa) and a posterior (ACp) arm (Figure 3), and bifunctional activity of the secreted semaphorin Sem3B is required for proper AC positioning and guidance (Julien et al. 2005). Sem3B mRNA is found in the subventricular zone lining the lateral ventricle, adjacent to ACa axon projections. Careful examination of Sem3B mutants reveals disorganization of ACa and ACp projections, with a significant increase in defasciculation and extension of ACa projections rostrally and laterally and an expansion of AC ventrally at the level of midline crossing (Julien et al. 2005). Sem3B repels ACp axons and attracts ACa in vitro. Interestingly, complexes that include both Npn-2 and the Ig superfamily member NrCAM can be immunoprecipitated from embryonic brain extracts. Association between Npn-2 and NrCAM is indeed required for Sem3B-mediated ACa attraction and ACp repulsion because both attraction and repulsion are inhibited in vitro by NrCAM function blocking antibodies, and NrCAM null mice display AC phenotypes similar to those observed in Sem3B or Npn-2 mutants. Although it remains to be determined if distinct holoreceptor composition contributes to differential Sem3B guidance of ACa and ACp axons, Sem3B-mediated activation of focal adhesion kinase (FAK) signaling selectively in neurons that contribute to ACa distinguishes Sem3B-mediated ACa attraction from ACp repulsion. Therefore, in both fish and mice, secreted semaphorin–mediated attraction is required to direct AC formation, and it will be important to define in vivo how attractive and repulsive responses to these cues are regulated.

Extrinsic modulation of growth cone attractive and repulsive responses to individual guidance cues ranges from the conversion of netrin-1 attraction to repulsion by laminin-1 (Höpker et al. 1999), to the regulation of ephrin-A2 contact–mediated repulsion by the metalloprotease kuzbanian (Hattori et al. 2000). Bifunctionality of the mammalian transmembrane class 5 semaphorin Sem5A is also mediated by extrinsic factors, in this case by interactions with chondroitin sulfate.
Secreted semaphorin attraction and repulsion of anterior commissure (AC) axons. Sema3B attractive and repulsive functions are required for proper projection of the AC in the mouse brain. Sema3B is expressed in the subventricular zone surrounding the lateral ventricle and signals through a holoreceptor complex that includes Npn-2 and NrCAM on anterior pars (ACa) axons to attract across the CNS midline and toward anterior piriform cortex and olfactory structures. Conversely, Sema3B exerts a repulsive effect on Npn-2/NrCAM-expressing posterior arm (ACp) axons, which project to the piriform cortex of the temporal lobe. Other abbreviations used: CC, corpus callosum; LV, lateral ventricle; OB, olfactory bulb.

**Figure 3**

Secrected semaphorin attraction and repulsion of anterior commissure (AC) axons. Sema3B attractive and repulsive functions are required for proper projection of the AC in the mouse brain. Sema3B is expressed in the subventricular zone surrounding the lateral ventricle and signals through a holoreceptor complex that includes Npn-2 and NrCAM on anterior pars (ACa) axons to attract across the CNS midline and toward anterior piriform cortex and olfactory structures. Conversely, Sema3B exerts a repulsive effect on Npn-2/NrCAM-expressing posterior arm (ACp) axons, which project to the piriform cortex of the temporal lobe. Other abbreviations used: CC, corpus callosum; LV, lateral ventricle; OB, olfactory bulb.

**Fasciculus retroflexus (fr):** a neural tract in the caudal diencephalon composed of axons that originate in the medial habenula and project ventro-caudally to the interpeduncular nuclei of the midbrain

**Prosomere 2:** one of several morphological divisions of the diencephalon

proteoglycans (CSPGs) (Kantor et al. 2004). Proteoglycan facilitation of guidance cue function is well known (Van Vactor et al. 2006), and recent observations show that neuronal localization of the class 3 semaphorin Sema3A is influenced by extracellular matrix proteoglycans (De Wit et al. 2005). However, CSPGs serve to switch Sema5A from being an attractant to a repellent for midbrain axons emanating from the medial habenula that make up the fasciculus retroflexus (fr) tract (Kantor et al. 2004). Sema5A is expressed in prosomere 2, a region in the developing midbrain that defines the rostral boundary along which the fr extends. CSPGs are also expressed in prosomere 2, and CS or Sema5A function blocking reagents cause fr axons to aberrantly extend into prosomere 2. Sema5A functions as a repellent for fr axons, but only in the presence of CSPGs. In the absence of CSPGs, Sema5A functions as an fr attractant, and indeed Sema5A is expressed on fr axons themselves, presumably promoting fr axon fasciculation and extension. CSPGs most likely serve to alter the conformation of Sema5A by associating with its thrombospondin type 1 (TSP) repeats, the signature domain of class 5 semaphorins, and freeing the Sema5A sema domain for interactions with a Sema5A repulsive receptor. In nonneuronal cells, Sema5A utilize PlexB3 as a repellent receptor (Artigiani et al. 2004). However, fr axons likely utilize a repulsive receptor other than PlexB3 (Kantor et al. 2004).
SEMAPHORIN-MEDIATED CELL MIGRATION

Neuronal Migration

Although semaphorins were initially identified as axonal repellents, it is now clear that they participate in a diverse spectrum of neuronal and nonneuronal cell migration processes and influence cortical, cerebellar, and cardiovascular development. For example, in the developing vertebrate brain, GABAergic interneurons originate in the medial ganglionic eminence in the basal telencephalon and migrate tangentially along well-defined pathways to the neocortex or the striatum, depending upon guidance cue receptor expression (Marin & Rubenstein 2003). Migrating interneurons destined to inhabit the cortex avoid the striatum. The secreted semaphorins Sema3A and Sema3F, which are expressed in the striatum, appear to prevent neuropilin-expressing interneurons from invading this region of the developing brain (Marin et al. 2001). In Npn-2 null mutant mice, a significant population of prospective Npn-2-positive cells are detected in the striatum. Therefore, class 3 semaphorins influence GABAergic neuronal migration as repellents, analogous to their role in restricting neuronal process extension and branching. Similarly, secreted semaphorins function to restrict neural crest migration. Sema3F expressed in the posterior sclerotome of developing somites repels the Npn-2-expressing neural crest cells, restricting them to the anterior sclerotome (Gammill et al. 2006). In both Npn-2 and Sema3F null mice, neural crest cells invade the posterior sclerotome and migrate as a continuous sheet of cells, and in vitro cultured neural crest cells avoid Sema3F. Secreted semaphorins serve a similar role in the regulation of neural crest migration in zebrafish (Yu & Moens 2005).

Moreover, recent work shows that, in addition to secreted semaphorins serving to repel migrating neurons from inappropriate locations, the transmembrane semaphorin Sema6A is required for a qualitatively distinct granule cell migration event in the cerebellar cortex (Kerjan et al. 2005). The developing postnatal cerebellar cortex consists of four major cell layers: the outermost transient external granule layer (EGL), the intervening molecular layer (ML), the Purkinje cell layer (PCL), and innermost inner granule cell layer (IGL) (Figure 4). Cerebellar granule cells are born in the embryonic rhombic lip and migrate tangentially over the cerebellar plate, ultimately to populate the EGL of developing cerebellum. After arriving at the EGL, granule cells exit the cell cycle and undergo a stereotyped pattern of axonal extension and soma migration. Granule cells first extend two horizontal processes laterally in opposite directions, called parallel fibers. This is followed by the extension of a third process perpendicular to the parallel fibers away from the pial surface, giving rise to the characteristic T-shaped granule cell morphology. Once this radial process is extended, the granule cell soma migrates inward along this process through the ML to reside in the expanding IGL. It is this final radial migration event that critically depends on Sema6A. Sema6A is transiently expressed in cerebellar granule cells just prior to their radial migration. In Sema6A mutant mice, tangential granule cell migration and extension of parallel fibers occur normally, but the final radial migration step is severely disrupted. Approximately 40% of granule cells in these mutants fail to complete their journey to the IGL and are found trapped in the ML. Sema6A function in granule cells is nonautonomous, indicating that Sema6A functions as a ligand, and not as a receptor, in this process (Kerjan et al. 2005). At least two mechanisms may account for Sema6A function in granule cell migration. Sema6A expressed in the deeper EGL may be involved in contact-mediated repulsion, guiding the nuclear/soma migration of granule cells away from the EGL. Alternatively, Sema6A may serve as a de-adhesion molecule, facilitating granule cell migration.
Wild type

Sema6A

ML EGL PCL

IGL

Sema6A

radial migration by preventing cellular attachments among granule cells. Whatever the precise mechanism, this work demonstrates that contact-mediated semaphorin repulsion regulates migration events critical for the establishment of cerebellar laminar organization, and future work will establish if class 6 semaphorin–mediated regulation of soma positioning utilizes signaling mechanisms distinct from those required for semaphorin repulsion of migrating neurons en route to their final destination.

Semaphorin-Mediated Cell Migration in Cardiac Development

Semaphorin-mediated cell migration events are crucial for heart development, employing both forward and reverse semaphorin signaling and a panoply of coreceptors to coordinate the complex migration events underlying cardiac morphogenesis. The expression of the transmembrane semaphorin Sema6D and its receptor PlexA1 can be detected in distinct endothelial and myocardial cell populations during early chicken and mouse
embryonic development, suggesting that they regulate migration events unique to each of these cell types. Indeed, loss-of-function studies demonstrate that Sema6D coordinates the narrowing and bending of the developing ventricular chamber by enhancing outgrowth and migration of the conotruncal (CT) segment and also by inhibiting the migration of cells that constitute the developing ventricle (Toyofuku et al. 2004a). Sema6D signals its repulsive effects on ventricular expansion through a Sema6D holoreceptor complex that includes PlexA1 and the coreceptor OTK. Sema6D attractive effects on CT segment cell migration, however, result from the use of a Sema6D holoreceptor complex composed of PlexA1 and the VEGFR2 RTK. Although PlexA1 is required as a receptor for Sema6D attraction and repulsion, OTK and VEGFR2 function solely to mediate ventricular repulsion or CT attraction, respectively, providing further evidence for the important roles that plexin coreceptors serve in determining whether semaphorin cues function as attractants or repellents.

In addition to coordinating endothelial-cell migration events in the developing heart, Sema6D also plays a central role in myocardial organization. Expansion of the compact layer of the developing ventricle, and also trabeculation (the migration of myocardial cells in the trabeculae orthogonal to the ventricular layer), depends critically on Sema6D forward and reverse signaling (Figure 5) (Toyofuku et al. 2004b). Sema6D and PlexA1 are both expressed in compact-layer cells; however, Sema6D is found in the trabeculae, and PlexA1 is expressed in endocardial cells. The absence of Sema6D or PlexA1 during chicken heart development results in a small and thin ventricular compact layer and defective trabeculation (Figure 5b). Remarkably, Sema6D serves as both a PlexA1 ligand and a receptor in its own right to orchestrate compact-layer expansion and trabeculation. This can be appreciated by the observation that the PlexA1 extracellular domain can rescue trabeculation defects resulting from siRNA knockdown of PlexA1 but not those resulting from knockdown of Sema6D. However, the PlexA1 extracellular domain does not rescue ventricular expansion defects resulting from PlexA1 knockdown. In combination with additional experiments, these results show that Sema6D functions as a receptor to direct myocardial cell migration and that Sema6D forward signaling is essential for ventricular expansion. The recruitment of PlexA1 to the Sema6D receptor results in the association of activated Abl tyrosine kinase with the Sema6D cytoplasmic domain. This in turn leads to the phosphorylation of the Ena/Vasp family member Mena and the release of this essential regulator of cell migration from the Sema6D cytoplasmic domain. Interestingly, accumulating evidence indicates that transmembrane semaphorin reverse signaling is a general property of these guidance molecules. In flies, the Sema-1a cytoplasmic domain has the capacity to regulate synapse organization and function (Godenschwege et al. 2002), Sema-1a is required cell-autonomously for the projection of photoreceptor cells to the optic lobe (Cafferty et al. 2006), and very recent results show that Sema-1a cell-autonomously directs dendritic targeting of olfactory projection neurons to glomeruli in the antennal lobe (Komiyama et al. 2007). In mammals, Sema4D likely serves as a receptor in the immune system (Kumanogoh & Kikutani 2004). Furthermore, Sema6A is expressed in mouse thalamic neurons and is required for the proper organization of thalamocortical projections, raising the possibility that, as in flies, transmembrane semaphorins function as guidance receptors in the mammalian nervous system (Leighton et al. 2001). Taken together, the evidence is clear that semaphorin signaling plays a central role in regulating cell migration in addition to cellular process morphology, and it will be interesting to determine the extent to which distinct and shared signaling events underlie these functions of semaphorins and their receptors.

**Migration**

Endocardium

**Ventricular expansion**

Wild type

Sema6D

siRNA

PlexA1

Abl kinase

Mena

Endocardium

**Trabecular layer**

**Compact layer**

**Forward signaling**

**Reverse signaling**

286 Tran • Kohlkem • Bharadwaj
THE SEMAPHORIN SIGNALING CASCADE

Extensive work on both neuronal and non-neuronal semaphorin guidance mechanisms has defined a myriad of intracellular signaling events crucial for semaphorin-mediated repulsive and attractive guidance functions (Kruger et al. 2005, Pasterkamp 2005). Recent work on this issue provides additional links to intracellular signaling pathways, sheds light on how semaphorin signaling dynamically regulates cytoskeletal organization, and provides a framework for understanding how semaphorin guidance events are integrated with the promotion of process outgrowth and cell attachment.

Repulsive guidance cues signal the remodeling of the actin meshwork within growth cones, and so it is no surprise that both negative and positive regulators of actin dynamics are downstream targets for these cues. Microtubules, which form the more stable central core of the growth cone and are prominent in axonal and dendritic shafts, are not passive followers in guidance decisions. Live-imaging studies demonstrate that a subset of dynamic microtubules transiently invade distal dynamic regions of the growth cone, strongly suggesting that such microtubules play an active role in responding directly to activated guidance cue receptors (Kalil & Dent 2005, Zhang et al. 2003). Supporting this idea is that signaling by axonal repellents and neurotrophins can directly regulate microtubule dynamics (Kalil & Dent 2004). In addition, the regulation of neuronal process attachment to extracellular components plays a key role in guidance cue function. Integrins, key mediators of adhesive cellular interactions, have thus emerged as important components of the signaling networks essential for guidance cue signaling events.

The Intersection between Plexin and Integrin Signaling

Plexins possess in their intracellular domains two highly conserved regions that share sequence similarity with Ras family–specific GTPase activating proteins (GAPs). A major breakthrough in defining the significance of these GAP homology regions was the demonstration that, upon binding its ligand Sema4D, the PlexB1 receptor is stimulated to function as an R-Ras GAP (Oinuma et al. 2004). This PlexB1 GAP activity requires association with a Rho family GTPase called Rnd1 that binds constitutively to the PlexB1 cytoplasmic domain between its two GAP homology domains (Figure 6). Rnd1-dependent R-Ras activation also follows ligand binding to PlexA receptors, strongly suggesting that downregulation of R-Ras signaling through the action of plexin R-Ras GAP activity is a common feature of plexin signaling. However, there is an added level of complexity in the regulation of PlexA GAP activity because it is dependent upon the activation of yet
Semaphorin intracellular signaling events. Semaphorin signaling results in the suppression of actin and tubulin dynamics and the inhibition of integrin-mediated adhesion to the extracellular matrix, leading to repulsion. Plexin-mediated effects on the actin cytoskeleton are mediated primarily through the modulation of Rho-GTPases. In contrast, the plexin R-Ras GAP (GTPase-activating protein) domain suppresses R-Ras activity and PI3 kinase (PI3K) signaling, leading to downregulation of integrin activation and the PI3K-Akt pathway. This schematic reflects signaling events highlighted in this review, but space considerations preclude depicting many additional semaphorin signaling components, including numerous kinases, cyclic nucleotides, and MICAL (molecule that interacts with CasL), and contributions by PlexA receptors to the inhibition of actin dynamics.

Another GTPase, the Rho-GTPase family member Rac, by a FERM domain–containing guanine nucleotide exchange factor (GEF) called FARP2 (Toyofuku et al. 2005). Under basal conditions FARP2 remains bound to PlexA, but upon Sema3A stimulation FARP2 dissociates from PlexA and activates Rac. Rac activation, through an as-yet- unidentified mechanism, primes the PlexA receptor for the recruitment of Rnd1 and subsequent stimulation of its R-Ras GAP activity (Figure 6).

These findings shed light on cross talk between repulsive and attractive signaling pathways because R-Ras can promote cell...
adhesion through integrin activation (Sethi et al. 1999, Zhang et al. 1996). Semaphorin signaling often facilitates de-adhesion. In endothelial cells, class 3 semaphorin signaling locally antagonizes integrin activation and the formation of focal adhesions (Serini et al. 2003), and class 4 semaphorins appear to function in a similar manner in fibroblasts (Barberis et al. 2004). Similarly, the viral semaphorin receptor PlexC1 inhibits integrin-mediated adhesion required for chemokine-dependent cell migration (Walzer et al. 2005). In PC12 cells, Sema4D activation of PlexB1 inhibits R-Ras, which in turn leads to the suppression of β1 integrin activity and cell migration on a collagen substrate (Oinuma et al. 2006).

Recent analysis of R-Ras signaling pathway components provides insight into how integrin and semaphorin signaling is coordinately regulated. R-Ras-mediated integrin activation requires PI3 kinase (PI3K), and expression of a constitutively active PI3K renders Sema4D unable to suppress integrin-mediated migration of COS cells and also makes hippocampal neurons insensitive to the repulsive effects of Sema4D-PlexB1 signaling (Ito et al. 2006, Oinuma et al. 2006). Therefore, plexin inhibition of integrin inside-out activation appears capable of facilitating semaphorin-mediated repulsion. An additional avenue by which semaphorin signaling appears to downregulate integrin activation results from the ability of FARP2, following Sema3A binding to PlexA1, to inhibit focal adhesion assembly (Toyofuku et al. 2005). It is interesting, in light of these observations that plexin receptor activation serves to inhibit inside-out integrin signaling, that the protein molecule that interacts with CasL (MICAL), a large cytosolic protein that associates with PlexA in Drosophila and is required for Sema1a-mediated repulsion, may provide a direct link between plexin signaling and the regulation of Cas family of signaling-adaptor proteins (Terman et al. 2002). Cas proteins are key signaling intermediates downstream of integrin receptors (Chodniewicz & Klemke 2004), and semaphorin signaling may also serve to antagonize Cas function and, therefore, outside-in integrin signaling.

Plexin Signaling Impacts on Microtubule Organization

The modulation of R-Ras activity by plexin signaling may also promote semaphorin repulsion through effects on microtubule dynamics. Sema3A repulsive signaling requires the downregulation of the PIP3 phosphatase PTEN, a negative regulator of the PI3K-Akt signaling pathway that generates the inactive phosphorylated form of glycogen synthase kinase-3 beta (GSK-3β) (Chadborn et al. 2006) (Figure 6). Consistent with this observation, GSK-3β is converted from an inactive serine-9 phosphorylated form to its active serine-9 unphosphorylated form upon Sema3A treatment (Eickholt et al. 2002). A GSK-3β substrate that is important for Sema3A repulsive responses in neurons is the collapsin response mediator protein (CRMP)-2. Disruption of CRMP-2 activity has long been known to block Sema3A repulsion (Goshima et al. 1995), and CRMP-2 associates with tubulin heterodimers and promotes microtubule polymerization (Fukata et al. 2002, Inagaki et al. 2001). In response to Sema3A, CRMP-2 is phosphorylated by GSK-3β and thereby inactivated. These phosphorylation events are critical for semaphorin signaling because the expression of CRMP-2 proteins harboring mutations in these GSK-3β phosphorylation sites, or treatment with GSK-3β inhibitors, renders neurons insensitive to Sema3A-mediated growth cone collapse (Brown et al. 2004, Eickholt et al. 2002, Uchida et al. 2005). Interestingly, PlexB1 R-Ras GAP activity is essential for Sema4D-induced dephosphorylation of Akt, activation of GSK-3β, and neuronal growth cone collapse (Ito et al. 2006). Therefore, attenuation of R-Ras by semaphorin-plexin signaling appears to have an inhibitory effect on both integrin-mediated cell adhesion and CRMP-mediated microtubule
polymerization, underscoring the importance of plexin receptor R-Ras GAP activity.

**Plexin Signaling Requires Rho-GTPases**

Given the well-established roles for Rho-GTPases in the modulation of the actin cytoskeleton, it is not surprising that plexin receptor activation regulates the activities of both Rho and Rac. However, the mode of Rho-GTPase regulation differs among various plexins. Given the ability of Rho to promote process retraction and that of Rac to facilitate extension, the observation that PlexB receptor activation activates Rho and inhibits Rac is satisfying. In vertebrates, PlexB1 activates Rho by recruiting a PDZ domain–containing Rho-GEF to its C terminus, whereas PlexB1 downregulation of Rac is brought about by the direct association of PlexB1 with GTP-bound, active Rac, sequestering Rac from its downstream effector, PAK (Pasterkamp & Kolodkin 2003). In *Drosophila*, PlexA employs a somewhat different molecular mechanism to inactivate Rac and activate Rho; however, PlexA-mediated effects on Rho-GTPases clearly play key roles in regulating cellular morphology in response to semaphorins. Given the importance of Rac-GTPases in promoting process outgrowth and cell migration, it is intriguing that Sema3A-PlexA-mediated collapse of neuronal growth cones as well as COS cells requires the activation of Rac (Jin & Strittmatter 1997, Turner et al. 2004). This is in contrast to the suppression of Rac activation by PlexA. PlexA-mediated activation of Rac appears to depend in part upon the Rac-GEF FARP2 and serves to activate PlexA R-Ras GAP activity. However, because Sema3A stimulates LIM-kinase-dependent phosphorylation of the actin-severing protein cofolin, which is required for Sema3A growth cone collapse (Aizawa et al. 2001), it remains to be seen if Sema3A-PlexA activation of Rac also contributes to LIM-kinase effects on cofolin and actin dynamics.

Analysis of plexin receptor function demonstrates that plexin receptors are part of a complex signaling network capable of dynamically regulating multiple cytoskeletal components in response to semaphorin guidance cues. Indeed, our focus here on more recent advances in our understanding of the semaphorin cascade fails to consider numerous kinases and additional signaling components that contribute to semaphorin signaling events in a variety of settings (Kruger et al. 2005, Yazdani & Terman 2006). Furthermore, cross talk between plexin and integrin signaling cascades demonstrates how multiple inputs to extending cellular processes and migrating cells can be integrated to allow for discrete responses to guidance cues. In addition to plexin signaling events that directly impact cytoskeletal dynamics, additional levels of regulation play key roles in facilitating semaphorin-plexin signaling. These include the regulation of local translation events required for repulsive responses to semaphorins (Campbell & Holt 2003, Wu et al. 2005), the intriguing possibility that semaphorin signaling regulates endocytosis and retrograde transport (Fournier et al. 2000, Togashi et al. 2006), and the modulation of semaphorin signaling by intracellular second messengers (Song et al. 1998, Terman & Kolodkin 2004).

**CONCLUSION**

Semaphorins and their receptors influence cellular morphology in a large variety of systems. We focus here on how this diverse family of guidance cues and its receptors influences the development of complex tissues, with an emphasis on neural and cardiovascular systems, to illustrate principles of semaphorin function. However, extensive work on semaphorin function in the immune system (Bismuth & Boumsell 2002, Takegahara et al. 2005), in cancer biology (Chedotal et al. 2005), and in the regenerating injured nervous system (Pasterkamp & Verhaagen 2006) demonstrates key roles for semaphorins following the completion of development. Our
current understanding of semaphorin function is built upon a foundation of principles governing how these cues sculpt developing neuronal and nonneuronal processes. It remains to be determined whether similar principles can be applied to the regulation of distinct aspects of cellular morphology, including cell migration, soma translocation, and process pruning and remodeling. However, genetic, cellular, and biochemical tools are now available to begin to address these and other issues. We can expect that experiments over the next several years will enhance our understanding of how semaphorins contribute to organogenesis and that the elucidation of underlying signaling events will define how cellular responses to multiple distinct families of guidance cues are integrated.

### SUMMARY POINTS

1. Semaphorin guidance cues regulate distinct aspects of neuronal process morphology through their regulation of axonal fasciculation, pathfinding, target selection, branching, and dendritic morphology and targeting.
2. Semaphorins regulate nonneuronal cellular morphology, including within the developing cardiovascular system.
3. Semaphorin-mediated repulsion and attraction both sculpt neuronal and nonneuronal process morphology.
4. Plexin receptors form holoreceptor complexes with a wide range of coreceptors to transduce specific semaphorin guidance signals.
5. Semaphorins direct select cell migration events during development.
6. Transmembrane semaphorins can also function as receptors in a wide variety of cell types.
7. Semaphorin-plexin signaling modulates cellular morphology through multiple signaling pathways that regulate cytoskeletal dynamics and that are integrated with other signaling cascades, including integrin signaling.

### FUTURE ISSUES

1. How plexin signaling cascades differ in neuronal and nonneuronal cells remains to be determined.
2. Postnatal semaphorin and plexin signaling contributions to adult synaptic function, neuronal regeneration, tumor metastasis, and immune system function are currently under intense investigation.
3. Understanding the signaling events downstream of transmembrane semaphorins functioning as receptors will shed light on numerous cellular interactions, including those crucial for cardiovascular development, the generation of olfactory system connectivity, and axonal pathfinding events during neural development.
4. Defining cross talk between the semaphorin signaling cascade and other signaling pathways downstream of guidance cues, growth factors, or integrin receptors will provide insight into how cells integrate distinct cues so as to allow for coherent alteration of cellular morphology.
5. In-depth analyses of existing and new mutations in the genes encoding semaphorins and their receptors will broaden our view of how this large family of guidance cues and their receptors participate in the development and maintenance of diverse organ systems.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank David Ginty, R. Jeroen Pasterkamp, and Yimin Zuo for helpful comments on this manuscript. We apologize to our colleagues for many sins of omission owing to space limitations. Work in the authors’ laboratory is supported by grants from the NIH (from NINDS to T.S.T. and A.L.K and from NIMH to A.L.K.) and from the Packard Center for ALS Research at Johns Hopkins (to A.L.K.). A.L.K. is an investigator of the Howard Hughes Medical Institute.

LITERATURE CITED


He Z, Tessier-Lavigne M. 1997. Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell 90:739–51


Yazdani U, Terman JR. 2006. The semaphorins. *Genome Biol.* 7:211


