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The Molecular Biology of Axon Guidance

Marc Tessier-Lavigne and Corey S. Goodman

Neuronal growth cones navigate over long distances along specific pathways to find their correct targets. The mechanisms and molecules that direct this pathfinding are the topics of this review. Growth cones appear to be guided by at least four different mechanisms: contact attraction, chemoattraction, contact repulsion, and chemorepulsion. Evidence is accumulating that these mechanisms act simultaneously and in a coordinated manner to direct pathfinding and that they are mediated by mechanistically and evolutionarily conserved ligand-receptor systems.

The remarkable feats of information-processing performed by the brain are determined to a large extent by the intricate network of connections between nerve cells (or neurons). The magnitude of the task involved in wiring the nervous system is staggering. In adult humans, each of over a trillion neurons makes connections with, on average, over a thousand target cells, in an intricate circuit whose precise pattern is essential for the proper functioning of the nervous system. How can this pattern be generated during embryogenesis with the necessary precision and reliability?

Neuronal connections form during embryonic development when each differentiating neuron sends out an axon, tipped at its leading edge by the growth cone, which migrates through the embryonic environment to its synaptic targets, laying down the extending axon in its wake (Fig. 1). Observations of developing axonal projections in vivo have revealed that axons extend to the vicinity of their appropriate target regions in a highly stereotyped and directed manner, making very few errors of navigation. They do so apparently by detecting molecular guidance cues presented by cells in the environment (*1*). Studies in the past two decades have provided a detailed understanding of the cellular interactions between growth cones and their surroundings that direct pathfinding, which we summarize in the first section of this review. Our understanding of the molecular biology of axon guidance is, however, much more fragmentary. Molecules implicated as guidance cues or as receptors for these cues are introduced in the second section. Many of these molecules have only recently been identified, and it seems likely that additional guidance cues and receptors remain to be discovered. Moreover, in most cases the precise guidance functions of candidate ligand-receptor systems in vivo are poorly understood. In the third section we discuss specific guidance decisions in which the roles played by some of these molecules are beginning to be defined. As will become apparent, despite the many gaps in our knowledge the picture that is starting to emerge is that pathfinding is directed by the coordinate action of multiple guidance forces that are mediated by mechanistically and evolutionarily conserved ligand-receptor systems. A considerable body of evidence supports these conclusions (*2*).

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Cellular Interactions That Guide Axons

The appearance that axons give of unerring navigation to their targets is all the more

remarkable given the relatively large distances (as much as several centimeters, or more than a thousand times the diameter of the cell body) that many axons must grow to reach their targets. In practice, however, this task is simplified by two features.

First, axon trajectories appear to be broken into short segments, each perhaps a few hundred micrometers long. Individual segments often terminate at specialized cells that form intermediate targets or "choice points" for the axons, presenting guidance information that enables the axons to select and to initiate growth along the next segment of the trajectory. The complex task of reaching a distant target is thus reduced to the simpler task of navigating each individual segment and choice point in turn.

In insects, some intermediate targets are made up of small clusters of "guidepost cells," ablation of which results in misrouting of axons that normally contact them (*3*). Usually, though, intermediate targets are composed of large groups of functionally specialized cells, like those at the midline of the nervous system (*4–6*). Growth cones that approach an intermediate target may slow their migration and assume a more complex morphology with more filopodia (that is, sensory protrusions), presumably the better to sample the environment (*2*). Axon growth, therefore, appears to be characterized by at least two types of cellular behaviors: simple linear growth along "highways," punctuated by more complex decision-making behaviors at intermediate targets (choice points), as axons switch from one highway to another.

A second feature that simplifies the wiring of the nervous system is that this process occurs in a stepwise manner. The first axons that develop navigate through an axon-free environment when the embryo is still relatively small, but most axons face an expanding environment criss crossed by a scaffold of earlier projecting axons. Many later developing axons travel along preexisting axon tracts (or fascicles) for at least some of their trajectory (Fig. 1), switching from one fascicle to another at specific choice points (*7*). This "selective fasciculation" strategy simplifies the assembly of large nervous systems like that of humans, in which axons extend to their targets in successive waves over a period of several months.

Four guidance forces. The realization that axonal trajectories are made up of shorter segments pushes the question of axon guidance back one step: How do axons navigate each short segment and choice point? Embryological, tissue culture, and genetic experiments indicate that axons respond to the coordinate actions of four types of guidance cues: attractive and repulsive cues, which can be either short-range or long-range (*8*) (Fig. 1).

Ramón y Cajal proposed over a century ago that axon guidance might be mediated by long-range chemoattraction, a process akin to the chemotaxis of motile cells, in which target cells secrete diffusible chemoattractant substances that attract axons at a distance (*9*) (Fig. 1). In vitro experiments, in which neurons cultured with target cells turn toward these cells, demonstrate the existence of several chemoattractants secreted by intermediate or final targets of axons (*10–12*). More recently, long-range chemorepulsion was demonstrated with the finding that axons can be repelled in vitro by diffusible factors secreted by tissues that these axons normally grow away from (*13*, *14*) (Fig. 1).

Axons can also be guided at short-range by contact-mediated mechanisms involving nondiffusible cell surface and extracellular matrix (ECM) molecules. Axon growth requires a physical substrate that is both adhesive and permissive for growth (many adhesive substrates fail to support axon growth) (*15*) (Fig. 1). This process of contact attraction has also been implicated in selective fasciculation, in which growth cones confronted with several preexisting axon fascicles select a specific pathway (*7*) (Fig. 1). Likewise, the contact repulsion of axons, akin to the contact inhibition of cell migration (*16*), has been extensively documented (*17*). Thus, axon growth can be channeled by a corridor of a permissive substrate bounded by repulsive cues that serve to hem in the axons (*18*, *19*) (Fig. 1). Local repulsive cues also can serve to block the forward progression of axons (*4*, *20*). The responses of growth cones to repulsive cues can range from simple deflection to axonal arrest, to more dramatic changes in which the growth cone collapses and retracts (*19*, *21*, *22*).

Although we focus here on the guidance of the primary growth cone at the tip of the growing axon, many neuronal connections are made by secondary (collateral) branches of axons, which form de novo from secondary growth cones sprouted along the axon shaft. Both the initiation and subsequent guidance of secondary growth cones appear to be directed by the same forces that guide primary growth cones (*12*, *23*).

Much of the current focus of cellular studies of axon guidance is to define the precise complement of forces acting to direct particular guidance decisions. As illustrated below, the guidance of axons over individual segments of their trajectories appears to involve the simultaneous operation of several, and in some cases possibly all four, of these guidance forces. Thus, an individual axon might be "pushed" from behind by a chemorepellent, "pulled" from afar by a chemoattractant, and "hemmed in" by attractive and repulsive local cues. Push, pull, and hem: these forces appear to act together to ensure accurate guidance. However, this well-engineered redundancy complicates experimental analysis of guidance mechanisms because perturbation of any one mechanism often has a limited effect.

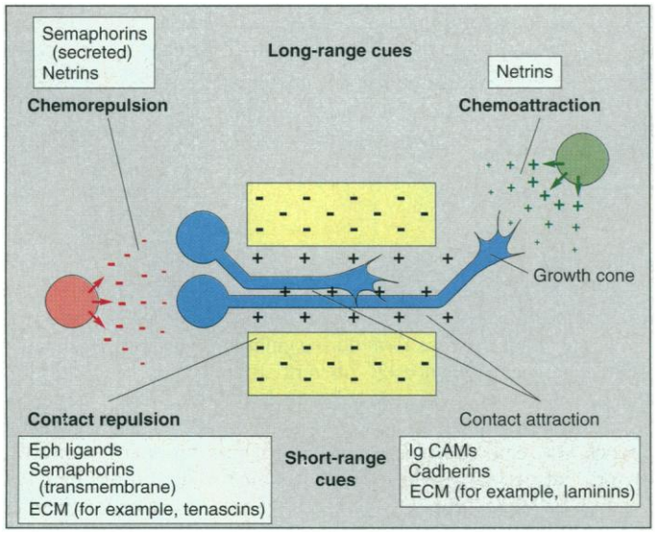

Fig. 1. Guidance forces. Four types of mechanisms contribute to guiding growth cones: contact attraction, chemoattraction, contact repulsion, and chemorepulsion. The term attraction is used here to refer to a range of permissive and attractive effects, and the term repulsion to a range of inhibitory and repulsive effects (*8*). Examples are provided of ligands implicated in mediating each of these mechanisms. There is not a one-to-one match between molecules and mechanisms because some guidance molecules are not exclusively attractive or repulsive, but rather bifunctional, and some families of guidance cues have both diffusible and nondiffusible members. Individual growth cones might be "pushed" from behind by a chemorepellent (red), "pulled" from afar by a chemoattractant (green), and "hemmed in" by attractive (gray) and repulsive (yellow) local cues. Axons can also be guided by cues provided by other axons (selective fasciculation). Push, pull, and hem: these forces act together to ensure accurate guidance.

Ligands and Receptors Implicated in Guidance

Given the evidence for four different guidance mechanisms, one might have expected

to find discrete classes of diffusible and nondiffusible factors, some attractive and others repulsive. Recent advances in identification of guidance cues have, however, blurred these distinctions. The first diffusible attractants to be identified, the netrins, are closely related to the laminins (Fig. 2B), nondiffusible ECM molecules (*24–27*). Similarly, the semaphorin family contains both cell-surface and diffusible members (Fig. 2C) implicated as short- and long-range repellents, respectively (*28–34*). In addition, several guidance molecules are bifunctional—attractive to some axons and repulsive to others. Such responses are presumably dependent on the receptors expressed by the growth cones (*14*, *35*, *36*).

Thus, there appears to be mechanistic conservation among guidance molecules, both short-range and long-range, and attractive and repulsive. In addition, both molecules and mechanisms appear to be ancient. In fact, evolutionary conservation of guidance molecules is so great that insights gained in invertebrates can be immediately relevant to vertebrates, and vice versa (*37*).

Cell adhesion molecules (CAMs) as ligands and receptors. Two large families of CAMs function during axon pathfinding: the immunoglobulin (Ig) and cadherin superfamilies (*38*). Many members of these two families can mediate homophilic adhesion, functioning as both a ligand on one cell and a receptor on another (*39*). Some members can also function as heterophilic ligands or receptors for distinct cell-surface or ECM molecules (*40*, *41*). Other apparently unrelated families of CAMs expressed in the nervous system include the Leucine-rich repeat (*42*, *43*) and Fasciclin I families (*44*). How many neural CAMs are encoded in any one genome is still unknown, although there are at least 10 in *Drosophila* and more than 50 in mammals. Many of these CAMs have signaling functions. Although some Ig CAMs contain cytoplasmic regions with protein tyrosine kinase or protein tyrosine phosphatase domains (*45*), most do not (Fig. 2A), despite their apparent roles as signaling receptors (*46*). Below we discuss experiments that implicate several Ig CAMs as receptors or ligands (or both) involved in pathfinding and fasciculation. Other CAMs for which important guidance roles have been indicated by in vivo studies include the Ig CAMs LAMP and IRREC (*47*). In addition, the phenotypes of mutations in the human *L1* gene are potentially consistent with L1 functioning in growth cone guidance (*48*).

Receptor protein tyrosine kinases (RPTKs). A variety of RPTKs modulate axon growth or regulate target invasion (Fig. 2A). In vertebrates these include fibroblast growth factor (FGF) receptors (*49*, *50*) and the Trk family of neurotrophin receptors (*51–53*), both receptors for secreted factors (discussed below). Neurotrophin receptors have also been implicated in regulating axonal branching (*51*, *54*). In *Drosophila*, the Derailed RPTK (related to vertebrate Ryk) has been implicated in regulating axon fasciculation (*55*). The largest subfamily of RPTKs in vertebrates is the Eph family, with over a dozen members; their ligands are all membrane-anchored via either a phospholipid anchor or a transmembrane domain (*56*, *57*). Many of the Eph receptors and ligands are expressed in the developing nervous system, and several of the lipid-anchored Eph ligands have recently been implicated as contact repellents that regulate axon fasciculation and topographic map formation [(*58–64*), discussed below], as well as in guidance to the target (*65*). In the case of transmembrane Eph ligands, recent evidence has raised the intriguing possibility of a role reversal, with the ligands functioning as receptors on axons and their "receptors" functioning as ligands that guide them (*66*).

Receptor protein tyrosine phosphatases (RPTPs). Genetic analysis in *Drosophila* has implicated several RPTPs in the control of axon fasciculation and defasciculation (*67*) (see below). Little is known about the ligands for RPTPs or their modes of activation. RPTPβ binds the Ig CAM contactin/F11, suggesting a link—possibly bidirec-

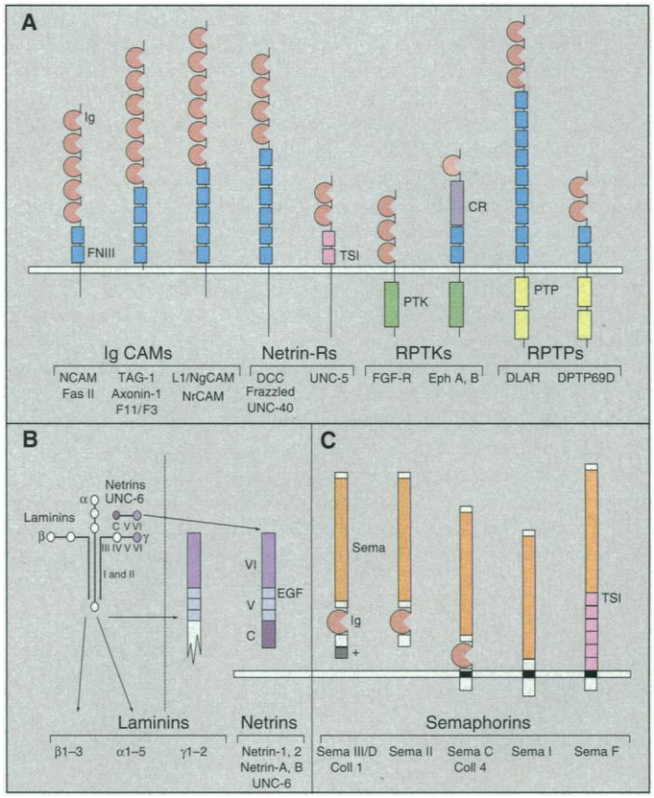

Fig. 2. Molecules that modulate axon growth. (**A**) Representatives of various subfamilies of the immunoglobulin (Ig) superfamily, including receptor protein tyrosine kinases (RPTKs) and receptor protein tyrosine phosphatases (RPTPs), that have been implicated as ligands or receptors (or both) in axon guidance (names shown are for those mentioned in the text). Some members of the Ig superfamily have extracellular domains possessing only tandem Ig domains, whereas others have both tandem Ig and fibronectin type III (FNIII) domains, or yet other motifs. For certain subfamilies, the first members were identified as proteins expressed on subsets of axons in the developing nervous system. For other subfamilies, the first members were identified in functional screens for adhesion molecules (CAMs). Yet other members (for example, UNC-40 and UNC-5) were identified as putative guidance receptors (the latter have longer cytoplasmic domains than CAMs). Some Ig superfamily members are linked to cell membranes by a GPI anchor. Many RPTKs and RPTPs implicated in axon guidance also have extracellular domains comprising tandem Ig domains or FNIII domains, or both. These subfamilies are highly conserved among vertebrates, insects, and nematodes. Ig, immunoglobulin domain; FNIII, fibronectin type III domain; TSI, thrombospondin type I domain; CR, cysteine-rich region; PTK, protein tyrosine kinase domain; PTP, protein tyrosine phosphatase domain. (**B** and **C**) The laminin, netrin, and semaphorin families of guidance molecules are conserved in structure and apparently in function among nematodes, insects, and vertebrates. (B) The laminins are heterotrimeric, cruciform glycoprotein complexes with constituent chains called α, β, and γ. There are at least five α, three β, and two γ chains in vertebrates. The netrins are related to the amino-terminal domains VI and V of laminin chains, although they then diverge from laminin sequences and are much shorter. (C) The semaphorins are a large family of cell-surface and secreted proteins. Most semaphorins are ~750 amino acids in length and share a common ~500–amino acid semaphorin domain; in several of these subfamilies, the semaphorin domain is followed by an Ig domain. One subfamily, however, contains members that are over 1000 amino acids in length; in these proteins, the semaphorin domain is followed by a set of tandem thrombospondin type I domains.

tional—between CAMs and RPTPs (68).

Extracellular matrix molecules and their receptors. Many ECM molecules, including the laminin (Fig. 2B), tenascin, collagen, and thrombospondin families, as well as fibronectin, vitronectin, and a variety of proteoglycans, can act either as promoters or inhibitors of neurite outgrowth and extension in vitro (69). Receptors for ECM molecules are predominantly integrins, Ig superfamily members, and proteoglycans (*41*, 69, *70*) (the latter may function primarily as binding or presenting molecules rather than as signaling receptors). Some proteoglycans might function as ligands to inhibit axonal extension (*71*). On the basis of their in vitro activities and in vivo expression patterns, many ECM molecules are expected to play roles in axon guidance, but little is known about actual guidance functions in vivo. In *Drosophila*, loss of laminin A function results in the stalling of a subset of sensory axons, implicating laminin as a permissive substrate for these axons (*72*). Similarly, interfering with integrin function in *Xenopus* retinal axons in vivo causes a foreshortening of the axons (*73*). In humans, mutations in the *KAL1* gene, which encodes a small ECM protein, cause defects that suggest a possible role for the *KAL1* gene product as a permissive substrate for olfactory axons (*74*).

Netrins and their receptors. The netrins are a small family of bifunctional guidance cues, capable of attracting some axons and repelling others (*24–27*, *75*) (see below). Netrins are proteins of ~600 amino acids related to the much larger laminins (Fig. 2B); they are diffusible, although the extent of their diffusion can be affected by interactions with cell surfaces or the ECM (*25*). Members of the DCC subfamily of the Ig superfamily (Fig. 2A) are components of receptors that mediate attractive effects of netrins (*76–78*). Genetic analysis in *Caenorhabditis elegans* has implicated UNC-5, a transmembrane protein that defines a distinct branch of the Ig superfamily (Fig. 2A), in mediating repulsive actions of the netrin UNC-6 (*79*) (Fig. 2B).

Semaphorins. The semaphorins are a large family of cell-surface and secreted proteins that appear to function as chemorepellents or inhibitors (*28–34*, *80*, *81*). The family is defined by a conserved ~500–amino acid extracellular semaphorin domain (*30*). There are at least five different subtypes of semaphorins, including secreted and transmembrane members (Fig. 2C). Nothing is yet known about the identity of semaphorin receptors. Vertebrate Collapsin-1/Semaphorin III/D is a potent inducer of sensory growth cone collapse (*29*) and has been implicated as a diffusible chemorepellent that patterns sensory axon projections in the spinal cord (*31*, *82*). In insects, semaphorins have been implicated in influencing steering decisions, inhibiting branching, and inhibiting formation of synaptic arbors (*28*, *34*), as discussed below. Recent evidence suggests that at least one semaphorin (Sema I) may also function as a contact attractant (*83*).

In Vivo Function of Guidance Molecules

The precise guidance roles of some of these molecules are beginning to be illuminated by functional analysis in vivo. Many of the recent insights into the molecular biology of axon guidance can be illustrated by referring to several examples: long-range guidance to intermediate targets, exemplified by guidance to and from the midline of the nervous system; complex decisions at intermediate targets, exemplified by guidance at the midline and by axon fasciculation and defasciculation; and target recognition.

Long-Range Guidance to and from the Midline

Structures at the ventral midline of the nervous system of organisms as diverse as nematodes, fruit flies, and vertebrates are important intermediate targets for many different classes of axons that navigate the midline along divergent trajectories (*4–6*) (Fig. 3). Axons that link the two sides of the nervous system project toward and across the midline, forming axon commissures. These commissural axons project toward the midline, at least in part, by responding to long-range chemoattractants emanating from the midline—the netrins (Fig. 2B). Netrins have an evolutionarily conserved role in guiding axons toward the ventral midline in nematodes, fruit flies, and vertebrates. In each organism, cells at the ventral midline express at least one netrin family member (Fig. 3), and loss of netrin function at the midline results in a misrouting of many axons and their failure to grow to the midline (*24–27*, *84*). The attractive actions of netrins appear to be mediated by receptor mechanisms involving members of the DCC subfamily of the Ig superfamily (Fig. 2A). Commissural axons express a DCC subfamily member (UNC-40 in *C. elegans*, Frazzled in *Drosophila*, and DCC in mammals), and loss-of-function analysis reveals defects similar to those observed in netrin knockouts (*76–78*, *85*). Furthermore, vertebrate DCC can bind netrin-1 and is required for the attractive function of netrin-1 in vitro (*77*). Some evidence suggests that DCC-related proteins may be only one component of attractive netrin receptor complexes (*76–78*).

How are netrins involved in guiding commissural axons? The simplest interpretation of the loss-of-function mutant phenotypes is that netrins function as instructive guidance molecules, attracting the axons toward the midline. Those data are, however, potentially compatible with a simpler role in which netrins are permissive for growth but do not provide directional cues. However, the findings that vertebrate commissural growth cones turn in vitro toward a source of netrin (*11*, *25*), that commissural axons in the mouse *netrin-1* knock-out give the appearance of wandering (*84*), and that ectopic pan-neural expression of netrins in

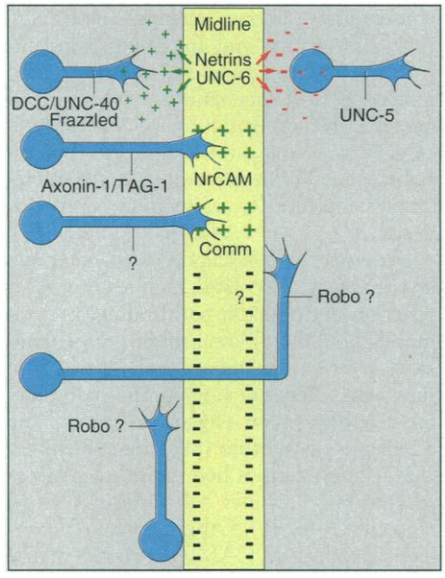

Fig. 3. Long-range and short-range guidance at the ventral midline. A composite picture of guidance at the midline drawing on mechanisms identified in nematodes, fruit flies, and vertebrates, at least some of which (and possibly all of which) are conserved among these organisms. The netrins appear to function as both long-range chemoattractants (green) and chemorepellents (red) for distinct classes of axons. Attraction of growth cones by netrins involves the DCC/UNC-40/Frazzled receptor (as shown in all three phyla), whereas repulsion of growth cones by netrins involves the UNC-5 receptor (as shown in *C. elegans*). In chick, crossing of the midline requires interaction of the Ig CAM axonin-1/TAG-1 on commissural axons with NrCAM on the surface of midline cells. In *Drosophila*, it also requires the midline expression of Commissureless (the growth cone receptor for Comm is at present unknown). Many commissural growth cones turn longitudinally along the midline after crossing. In *Drosophila*, the phenotype of *robo* mutants, when coupled with recent molecular data (*93*), is consistent with the hypothesis that axons express the putative Robo receptor that appears to function as a repulsive receptor for an unknown contact-mediated repellent at the midline, thus preventing these growth cones from recrossing the midline.

Drosophila results in commissural axon misrouting (*26*, *27*) provide evidence that a precise spatial distribution of netrins is important for correct directional growth in vivo.

Netrins also act as repellents for some axons that grow away from the midline. In *C. elegans*, mutations in the gene encoding the netrin UNC-6 impair not just ventrally directed migrations but also dorsally directed migrations away from the source of UNC-6 (*24*), suggesting that UNC-6 functions to repel these axons. Similarly, in vertebrates netrin-1 can repel trochlear motor axons, which normally grow dorsally away from a source of netrin-1 (*14*). The Ig superfamily member UNC-5 (Fig. 2A) is implicated in mediating the repulsive actions of UNC-6 on dorsally directed axons, because (i) *unc-5* functions cell autonomously in these cells, (ii) mutations in *unc-5* impair dorsal migrations to the same extent as mutations in *unc-6* (but in this case without affecting ventral migrations), and (iii) ectopic expression of *unc-5* in neurons that normally extend axons longitudinally causes their axons to project dorsally in an *unc-6*–dependent fashion (*79*). Thus, UNC-5 is part of a receptor mechanism that mediates migrations away from sources of UNC-6. The DCC homolog UNC-40 is also expressed by dorsally migrating axons, and mutations in *unc-40* also impair dorsal migrations, although to a much more limited extent than in *unc-5* mutants (*24*, *76*), suggesting that UNC-5 and UNC-40 might form a receptor complex. There is, similarly, evidence that other receptors involved in axon growth on some Ig CAMs are heteromeric complexes of Ig superfamily members (*86*).

Studies on netrin function also provide some of the clearest evidence for redundancy of guidance cues. Two apparently redundant netrins are coexpressed at the *Drosophila* midline (*26*, *27*). Moreover, when midline netrins or netrin receptors are genetically removed in nematodes, fruit flies, or vertebrates, the mutant phenotypes are only partially penetrant (for example, some commissural axons still reach the midline). Thus, other cues, likely including other diffusible signals secreted by midline cells (*84*, *87*), work in concert with the netrins to guide axons toward and away from the midline.

Complex Decisions: Local Guidance at the Midline

Once at the midline, growth cones make a variety of decisions (Fig. 3). Some never cross the midline, but most do. Some of those that cross subsequently continue to extend away from the midline, whereas most turn to project longitudinally, growing along or near the midline. Axons that cross the midline once, however, do not cross the midline again, despite navigating in the vicinity of other axons that are crossing. Thus, there may be at least two classes of local guidance cues: cues that allow certain growth cones to cross the midline and cues that prevent growth cones from either ever crossing the midline or from recrossing after their initial passage.

Studies in the chick embryo (*88*) have implicated two Ig CAMs in enabling axons to cross the midline: axonin-1 and NrCAM (Fig. 2A). Commissural axons and growth cones express axonin-1, whereas cells that form the midline (floor plate cells) express NrCAM (Fig. 3). These two Ig CAMs can bind heterophilically (*89*). Administration of reagents that perturb the axonin-1–NrCAM interaction in vivo in chicken embryos results in pathfinding errors of the commissural growth cones such that up to 50% of the axons fail to cross the midline and instead turn to travel along the ipsilateral border of the floor plate (*88*). Furthermore, commissural axons in vitro normally will grow onto floor plate cells, but stall or collapse on contact with these cells in the presence of reagents that block the axonin-1–NrCAM interaction (*90*). These experiments suggest that floor plate cells express an inhibitory factor on their surface whose function is normally masked by NrCAM, which is detected by a growth cone receptor involving axonin-1.

What is the function of this midline inhibitor? A likely role would be to prevent commissural axons from recrossing the midline after their first crossing. If so, then axons must acquire responsiveness to the inhibitor during or after crossing. This could be achieved by down-regulation of axonin-1 expression [as is observed in rat (*91*) but not chick (*88*)] or function, or by up-regulation of the expression or function of a receptor for the midline inhibitor. Studies in *Drosophila* provide evidence for the latter mechanism. In *roundabout* (*robo*) mutants, many growth cones that normally extend only on their own side instead now project across the midline, and axons that normally cross the midline only once instead cross and recross multiple times (*92*). *robo* encodes a transmembrane protein that functions cell autonomously in commissural neurons, consistent with the possibility that it is part of a receptor mechanism for a midline repellent (*93*).

Mutations in the *Drosophila commissureless* (*comm*) gene have the opposite phenotype, because commissural growth cones initially orient toward the midline but then recoil and do not cross it. *comm* encodes a

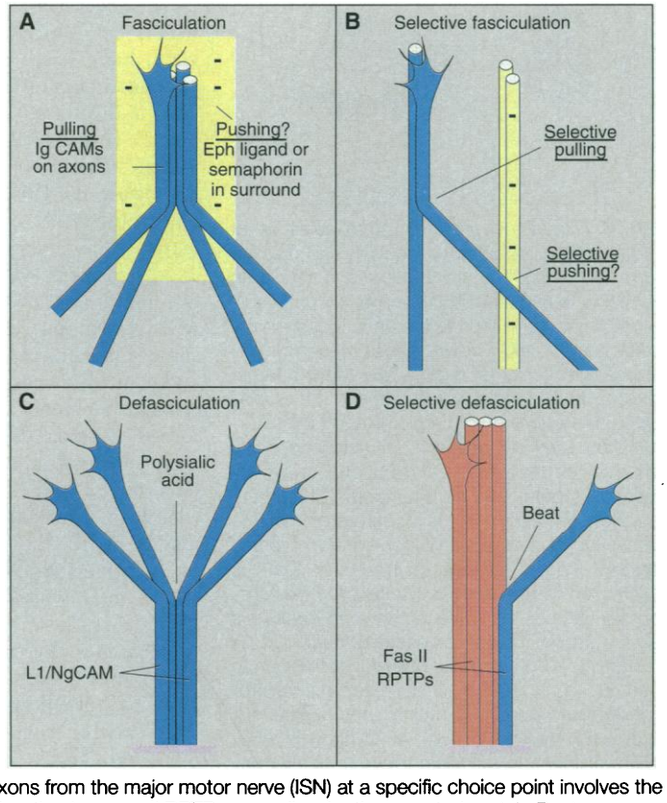

Fig. 4. Molecules that mediate fasciculation and defasciculation. (**A** and **B**) Axonal fasciculation appears to depend on a balance of attraction and repulsion. Ig CAMs such as Fasciclin II or L1/NgCAM on subsets of axons can function to "pull" axons together. Recent experiments also suggest that repulsive signals (possibly Eph ligands or transmembrane semaphorins) on surrounding cells or other subsets of axons can create an inhibitory environment that "pushes" axons together. (**C** and **D**) Mechanisms that regulate defasciculation. (C) Polysialic acid can drive the defasciculation of motor axons in the chick embryo, apparently by interfering with axon-axon adhesion mediated by the Ig CAM L1/NgCAM. (D) In *Drosophila*, defasciculation of SNb motor axons from the major motor nerve (ISN) at a specific choice point involves the modulation of Fasciclin II function by several RPTPs, as well as by the secreted protein Beat.

protein expressed by central nervous system (CNS) midline cells that lacks a signal sequence, has a transmembrane domain, and copurifies with membranes (*94*). As commissural growth cones contact and traverse the CNS midline, Comm protein is apparently transferred from midline cells to commissural axons. What is the function of Comm? One clue is derived from the observation that double mutants of *comm* and *robo* display a *robo*-like phenotype. Thus, although Comm is normally essential for axons to cross the midline, in the absence of Robo it is not at all required for crossing. One of several interpretations of these results is that Comm normally antagonizes the effects of the midline inhibitor sensed by Robo, a function not unlike that postulated for NrCAM at the midline of the vertebrate CNS. It is not known whether Comm, like NrCAM, has adhesive or attractive properties on its own.

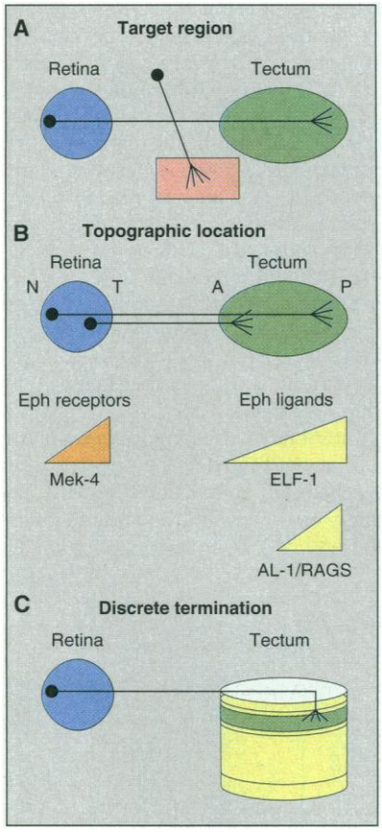

Fig. 5. Target recognition involves selection of target region, topographic location, and discrete termination site. The steps involved in finding an appropriate target are illustrated for the projection of retinal ganglion cells to the optic tectum in the chick embryo. (**A**) Growth cones recognize and invade specific target regions. (**B**) Within a target region, like the optic tectum, growth cones may be guided to their topographically appropriate termination sites by gradients of guidance cues. Thus, axons from nasal (N) retina project to posterior (P) tectum, and from temporal (T) retina to anterior (A) tectum. In the chick tectum, Eph ligands function as repellents for retinal axons and are expressed in gradients on the tectum. ELF-1 is expressed in an increasing anterior-to-posterior gradient across the entire tectum, and RAGS in a similar gradient across the posterior portion of the tectum. The Eph receptor Mek-4, which binds to both ELF-1 and RAGS, is expressed in a reciprocal gradient across the retina, with highest expression in the temporal retina. (**C**) Growth cones are also able to select discrete targets. In the chick embryo, retinal growth cones select a specific laminar termination site from among 16 laminae.

These studies on local guidance at the midline illustrate two points: (i) growth cones can be simultaneously exposed to a plethora of attractive and repulsive cues, and (ii) their complex behaviors might reflect a tight regulation of their responsiveness to these cues, including in some cases changes in the expression or function of guidance receptors as the axons progress forward (*91*, *95*, *96*).

Complex Decisions: Regulation of Axon Fasciculation

Growth cones often extend along the surface of other axons in axon fascicles and exit these fascicles to initiate the next leg of their trajectory. We have only recently begun to understand the complexity of mechanisms involved in regulating the initiation of fasciculation and defasciculation.

Molecules that pull axons together. CAMs, which can mediate cell-cell adhesion in vitro, have been implicated in mediating axon fasciculation in vivo (Fig. 4, A and B). This is illustrated by the analysis of Fasciclin II (Fas II) (*97*, *98*), an Ig CAM in insects related to vertebrate NCAM. In *Drosophila*, Fas II is expressed on a subset of embryonic CNS axons, many of which selectively fasciculate in three longitudinal axon pathways (*98*). In *FasII* loss-of-function mutants, these axons fail to fasciculate, whereas ectopic expression of Fas II on subsets of axons can prevent defasciculation and can also cause pathways that should remain separate to become abnormally joined together (*99*). In vertebrates, antibody perturbation studies have also indicated a role for Ig CAMs in axon fasciculation (*88*, *100*). Molecules other than Ig CAMs may be involved in regulating the initiation of selective fasciculation, as suggested by studies in *Drosophila* on the RPTK Derailed (*55*).

Molecules that push axons together. The function of CAMs on axons can be modulated by both positive and negative influences in the environment. If the environment provides a favorable substrate, the axons may prefer to grow on that substrate; lacking such a substrate, the axons might prefer to grow on each other (*2*). However, the extent of fasciculation may reflect not only the relative balance of attractive forces, but also the action of inhibitory factors. An example of this is provided by Sema I, a transmembrane semaphorin expressed on stripes of epithelial cells in the grasshopper limb bud. When Sema I function is blocked by antibodies, a pair of axons that are normally highly fasciculated when they grow on a stripe of Sema I instead defasciculate and branch (*28*). Although Sema I could affect fasciculation in several ways, one possibility is that Sema I is a negative factor that makes the substrate less favorable and drives the axons to fasciculate, a model supported by the finding that other semaphorins have repellent activities (*29*, *31*, *32*, *34*). Another example is provided by AL-1, a glycosyl phosphatidylinositol (GPI)–linked ligand for Eph receptors (Fig. 2A). In culture, vertebrate cortical neurons growing on astrocytes express a receptor for AL-1, whereas the astrocytes express this ligand (*58*). Cortical axons normally fasciculate in such cultures, but when AL-1 function is blocked, the axons defasciculate, suggesting that AL-1 is a repellent for cortical axons, making the astrocytes a less attractive substrate and thus driving fasciculation. This model is supported by the demonstration that AL-1 has collapse-inducing activity for cortical axons (*59*). These studies imply that the expression of molecules that create an inhibitory environment can push axons together. Thus, fasciculation may be like other types of guidance events in that it appears to be regulated by a balance of attraction and repulsion (Fig. 4A); it is tempting to speculate that selective fasciculation is mediated by differentially distributed attractive and repulsive ligands (Fig. 4B).

Molecules that drive defasciculation. If fasciculation is determined by the balance of attractive and repulsive forces on the axons relative to their surrounding environment, then defasciculation presumably involves a shift in the balance of these forces such that growth on nonaxonal substrates is now favored. In the examples discussed below, the expression of major axonal CAMs is maintained during defasciculation while other factors shift the balance of forces in favor of defasciculation.

Studies in the chick implicate polysialic acid (PSA), a carbohydrate that is covalently attached to the Ig CAM NCAM, as an important regulator of axon defasciculation (*101*) (Fig. 4C). Motor axons exit the CNS and are tightly fasciculated and intermingled as they reach the base of the limb bud. There they begin to defasciculate and to sort out into different axon pathways. This defasciculation appears to be

caused by a concomitant increase in levels of PSA found on these axons, because enzymatic removal of PSA impairs the defasciculation and causes an increase in projection errors (*101*). There is evidence that the addition of highly charged PSA chains to NCAM on a cell creates a charge cloud that sterically interferes with the ability of both NCAM and other neighboring CAMs on the cell to mediate adhesion (*102*). The effects of PSA removal on motor axons can be reversed by addition of antibodies to L1/NgCAM (*101*), a CAM expressed by these axons, suggesting that PSA normally functions to decrease L1/NgCAM-mediated axon fasciculation, increasing the ability of motor axons to defasciculate (*103*).

PSA is found only in vertebrates, where it is associated with only a subset of defasciculation events. Insights into other factors regulating defasciculation come from genetic studies on the peripheral projections of motor axons in *Drosophila*. The motor axons of the segmental nerve b (SNb) initially follow the intersegmental nerve (ISN) but then defasciculate from the ISN axons at a specific choice point and form a separate bundle that steers away (*104*). The Ig CAM Fas II is normally expressed at high levels on motor axons throughout their trajectories and is required to mediate their fasciculation (99, *105*). When the levels of Fas II on the axons are increased transgenically, the SNb axons fail to defasciculate at this choice point (*105*), suggesting that the selective defasciculation of motor axons requires modulation of Fas II function independent of changes in its expression.

Five genes have been identified that encode candidate negative regulators of Fas II function, as loss-of-function mutations in these genes give SNb defasciculation phenotypes similar to those observed when Fas II levels are increased (Fig. 4D). Three RPTPs (Dlar, DPTP69D, and DPTP99A) are expressed on motor axons, and mutations in the genes encoding them (either singly or in combination) give partially penetrant defasciculation phenotypes (*67*). Single mutations in two other genes—*beaten path* [*beat* (*104*, *106*)] and *sidestep* [*side* (*107*)]—result in similar but more highly penetrant phenotypes: virtually all SNb axons fail to defasciculate and instead continue extending along the ISN. *beat* encodes a secreted protein expressed by motoneurons, and genetic interactions between *beat* and *FasII* suggest that secretion of Beat by motor axons causes a decrease in adhesion of SNb axons to ISN axons (but not to other SNb axons) (*106*).

These studies are beginning to identify some of the molecules that regulate selective defasciculation, but their modes of action remain unknown. For example, it is not known whether RPTP function in defasciculation requires ligand binding. The secreted protein Beat might function to selectively decrease the attractiveness of some axons to others or modulate fasciculation in some other way. In addition, all of these molecules are made by the motoneurons themselves, and it is not known what environmental signals trigger the defasciculation.

Target Selection

Once at the target, growth cones invade the target region, where they often form a topographic projection pattern before selecting appropriate synaptic partners within the target field (Fig. 5).

Invading the target region. Evidence is mounting that invasion of the target region is regulated by both pathway- and target-derived cues. Target invasion can be regulated by members of the nerve growth factor (NGF) family of neurotrophins. For example, sympathetic innervation of the pineal gland and external ear is controlled by neurotrophin 3 (NT3), a factor made by these targets. In $NT3^{-/-}$ mice, sympathetic fibers approach but fail to invade these targets, a defect that can be rescued by the addition of exogenous NT3 (*53*). Similarly, invasion of other targets requires an increasing gradient of target-derived NGF (*52*). Evidence also exists for what appears to be the opposite type of mechanism. Retinal axons that project to the tectum in *Xenopus* travel along a path marked by FGF, which terminates abruptly at the target. When FGF is added exogenously to alter the gradient, axons fail to invade the target and instead skirt it; the same result is obtained when FGF function is blocked by expression of a dominant-negative FGF receptor in the axons (*50*). This result—that a failure to invade the target can be produced by either increasing or decreasing FGF function—suggests that the axons must be "primed" for target invasion by the detection of a downward gradient of FGF, although other interpretations are possible. These "upward" and "downward" gradient mechanisms are not mutually exclusive, and it remains to be seen whether such mechanisms operate generally to regulate target invasion.

Generating topographic projections. Topographically organized patterns of neuronal connections, in which neighboring neurons project to neighboring sites in the target, occur throughout the nervous system. The best studied example of the development of topographic projections is in the vertebrate visual system. Neighboring ganglion cells in the retina connect to neighboring target neurons in the optic tectum (or superior colliculus), thus projecting the retina's map of visual space as a topographic map across the tectum (Fig. 5B). Classic experiments by Sperry and others on the development and regeneration of this projection showed that axons that are experimentally deflected to inappropriate regions of the tectum can nonetheless reorient and home in on their topographically appropriate target region (*108*). Thus, the establishment of this pattern of projections appears to involve the recognition of positional information on the tectum.

The nature of this positional information has long fascinated neurobiologists. Sperry (*109*) argued against the idea that each axon has a unique label that is complementary to another unique label on its appropriate target cell, both because of the implausibly large number of labels that would be required and because this model does not provide a mechanism for each axon to find its target, except by wandering aimlessly around the tectum. These considerations led Sperry to propose that positional information might instead be encoded in the form of gradients of signaling molecules along both the anterior-posterior (AP) and dorso-ventral (DV) axes of the target, and that these gradients could be detected by complementary gradients of receptors on the axons. Positional information could thus be specified with a small number of molecules, and all axons could read positional information at every point on the tectum.

How might such gradients work to establish topography (*110*)? In principle, topographic projections could be directed by just one ligand gradient and one receptor gradient (along each of the AP and DV axes). This mechanism requires, however, that each axon seek out a specific concentration of ligand (a "set point," determined by the level of receptor expression) and migrate down-gradient at higher concentrations and up-gradient at lower concentrations to reach the set point (*57*). In this set-point model, the ligand acts sometimes as an attractant for the axon and sometimes as a repellent. An alternative class of models makes use of the antagonistic effects of two ligand gradients (along each axis). For example, an axon that is exposed only to an attractant gradient along a particular axis will tend to migrate all the way up the gradient, but if it is simultaneously exposed to a repellent gradient that starts shallow but becomes steep, it will migrate to that point along the axis where the repulsion precisely balances out the attraction. It is a relatively straightforward task to make axons originating from different positions on the retina project to different locations along the axis by making their responses to, for instance, the repellent gradient de-

pendent on their position of origin (*110*). In these "antagonistic gradient" models, unlike set-point models, the ligands can be pure repellents or attractants, thus invoking mechanisms similar to those discussed earlier in the context of other guidance decisions.

With the identification of gradients of repellent ligands for Eph receptors in the chick retinotectal system (Fig. 5B), the evidence, although very incomplete, has started to favor antagonistic-gradient models over set-point models. In vitro studies first established the existence of a repellent activity for retinal axons in tectal membrane preparations (*111–113*). This activity is present in an increasing anterior-to-posterior gradient in the tectum (*112*), and smooth gradients of the activity can repel the axons in vitro (*114*). Surprisingly, the specificity of the activity is not exactly as expected. Rather than showing graded responses, as would be expected according to antagonistic-gradient models, retinal axons fall into two classes: temporal retinal axons are all equally repelled and nasal axons are not repelled (*112*). Two related Eph ligands, RAGS (the chick homolog of AL-1, discussed above) (*60*) and ELF-1 (*61*), have since been found in overlapping anterior-to-posterior gradients across the chick tectum (Fig. 5B) and are candidates for repellents involved in topographic map formation. ELF-1 repels temporal axons without effect on nasal axons, both in vivo (*62*) and in vitro (*62, 63*), apparently affecting all temporal axons equally (*63*). Thus, ELF-1 appears to have the properties of the repellent activity associated with tectal membranes. In contrast, retinal axons are all repelled by RAGS in vitro (*60*), but there appears to be a smooth gradient of sensitivity of retinal axons across the AP axis, with temporal axons more sensitive than nasal axons (*63*), as postulated by antagonistic-gradient models.

Many questions are raised by these initial studies on Eph ligands. (i) Why are there two ligands, and what are their precise functions? The properties of ELF-1 could be consistent with a primary role in preventing temporal axons from entering the posterior tectum (*115*), whereas RAGS could in principle help axons in the posterior-most tectum find their precise targets. Loss-of-function studies will help clarify these points. (ii) What receptors are responsible for graded axonal responses, and how do such closely related ligands trigger such distinct responses? Several Eph receptors for these ligands on retinal axons have been identified, including one that is present in a gradient across the retina (*61, 63*) (Fig. 5B), but their contributions to the axonal responses are not known. (iii) What other factors work with Eph ligands to direct map formation? In particular, is there an attractive gradient along the AP axis of the tectum as well, as predicted by antagonistic-gradient models? (iv) Are Eph ligands involved in topographic map formation outside the retinotectal system? Evidence already exists for their involvement in directing topographic projections of hippocampal neurons to the septum (*64*).

Selecting discrete targets. After reaching their topographically appropriate sites along the DV and AP axes of the tectum, retinal axons turn to seek their appropriate laminar termination site within the tectum, which they select precisely from among 16 different laminae (Fig. 5C), presumably in response to laminar-specific guidance cues (*116*). The molecular basis of such discrete target selection is poorly understood, but some insights into the problem of target selection in general have been obtained from analysis of neuromuscular specificity in insects. In each abdominal hemisegment in *Drosophila*, ~40 motor axons select specific muscles from among 30 potential targets. Muscle ablation and duplication experiments indicate that individual axons can pick out their appropriate muscle targets with great precision (*117*). To date, the strongest candidates for targeting molecules are the two *Drosophila* Netrins, which are expressed by overlapping subsets of muscles (*26*). Embryos carrying a deletion of both genes—as well as embryos mutant in the *frazzled* gene, which is thought to be required for Netrin function—show partially penetrant defects in the projections of motor axons that normally innervate the Netrin-expressing muscles (*26, 78*). Ectopic expression of either *Netrin* gene in all muscles results in aberrant motor projections, particularly of those axons that normally innervate Netrin-expressing muscles. Thus, the Netrins appear to function as part of the normal targeting system for the motor axons that innervate the Netrin-expressing muscles.

There are, however, only two *Netrin* genes known in *Drosophila*, and they are expressed by only 4 of the 30 muscles, indicating that other types of molecules must work with the Netrins to control targeting. Genetic screens thus far have failed to uncover other genes that encode targeting ligands or receptors in this system (*104, 107*). Taken together with the partial penetrance of the *Netrin* mutant phenotype, this result suggests that discrete target selection might involve multiple redundant target labels, a possibility further supported by studies on *Connectin* and *FasIII* (which encode membrane-anchored CAMs) and *SemaII* [which encodes a secreted semaphorin (Fig. 2C)]. These genes are expressed by distinct subsets of muscles and may encode ligands involved in targeting, because when expressed ectopically in inappropriate muscles, they can attract (Fas III and Connectin) or repel (Sema II and Connectin) specific subclasses of motor axons (*34, 36, 118*). However, loss-of-function mutations in these genes do not individually result in obvious misrouting phenotypes, suggesting that they function in redundant recognition systems.

Conclusions

Our understanding of growth cone guidance mechanisms has progressed significantly over the past decade (*119*), and compared to just a few years ago (*1*), we now know a great deal more about the molecular mediators of axon guidance. At the same time, given the bewildering array of ligand and receptor mechanisms implicated in axon guidance that are being identified at an ever-increasing pace, one might be forgiven for thinking that the identification of so many different types of molecules confuses as much as it illuminates. Have any unifying themes started to emerge?

A first general theme is that axons appear to be guided through the combined operation of four guidance mechanisms (short- and long-range attraction, and short- and long-range repulsion), and that the outcome of any particular guidance decision appears to reflect the balance of attraction and repulsion operating at the decision point. Furthermore, based on in vivo analysis, these mechanisms appear to operate in all types of decisions—linear growth, sharp turns, axon fasciculation and defasciculation, and target invasion and selection. A further unification in our understanding appears to be emerging with the identification of molecules mediating these four guidance mechanisms and the discovery that the four mechanisms are mechanistically related and phylogenetically conserved. In fact, the findings that molecules that function as long-range attractants or repellents (netrins and semaphorins) are structurally related to molecules that function as short-range attractants and repellents (laminins and other semaphorins) suggest that long-range guidance molecules may have evolved from their short-range counterparts. This conclusion is further reinforced by the recent discovery that receptors implicated in mediating attractive and repulsive actions of the netrins are members of the Ig superfamily and are therefore close relatives of Ig superfamily members that are receptors (and ligands) implicated in several short-range guidance events, as well as in axon fasciculation. In addition, parallels between pathfinding events in nematodes, insects, and vertebrates illus-

trate vividly the evolutionary conservation in guidance mechanisms.

Although this convergence simplifies our understanding, at the same time there does not yet appear to be any overriding logic of how guidance molecules are used. Thus, Eph ligands, semaphorins, and netrins apparently assist in tasks as diverse as channeling growth, regulating fasciculation, and selecting specific targets. The situation is most vexing in the case of discrete target recognition, where one might have expected discrete targets to be labeled by some obvious scheme, for example, on the basis of different members of a gene family or alternatively spliced forms of a particular gene. Instead, what has emerged from the initial analysis of neuromuscular recognition in insects is the possibility that the remarkable specificity of discrete target selection might be directed by a patchwork of structurally disparate and functionally redundant guidance molecules, both attractive and repulsive, that have been cobbled together according to no obvious logic. Is there a deeper logic of target recognition that eludes us? It is too early to tell.

Another cautionary note relates to the functional redundancy of guidance mechanisms. Some of the redundancy, including the coordinate operation of the four canonical guidance forces, is presumably present to ensure a high degree of fidelity of axonal projections. There are, however, also examples of what might be termed "gratuitous redundancy," in some cases arising from gene duplications, like the presence of functionally redundant *Netrin* genes at the midline in *Drosophila*. Although redundancy is clearly present, it is worth pointing out that some of our worst fears about redundancy have not been borne out. Historically, studies of axon guidance progressed in the 1980s from an initial identification of candidate guidance molecules (often based on distribution and in vitro activities) to functional perturbations of these candidates. In many cases, strong phenotypes were not observed. This raised the fear that guidance mechanisms might be sufficiently overspecified to make it all but impossible to pinpoint the guidance function of any particular molecule. More recent studies indicate that this is not always true. Many guidance molecules have now been identified, mutations in which display a range of pathfinding and targeting phenotypes from dramatic to only partially penetrant. These studies have given us hope that an understanding of guidance mechanisms might be within reach.

What are some of the immediate challenges for studies of axon guidance? First, it is necessary to identify more guidance cues and receptors, as well as more factors that modulate the function of these effectors. The concern here is not to draw up an exhaustive list, but rather to determine what other major families of effectors and modulators function with those already identified and whether all guidance cues fit into the four canonical categories. Second, much more work is needed to determine the functions of these molecules in vivo. We still have a limited understanding of the precise functions of Ig CAMs, netrins, semaphorins, and Eph ligands, let alone less well characterized factors like Beat, Comm, and phosphatases. A major lesson in recent years is that elucidating the function of a candidate guidance cue requires identification not just of the cue but also of its receptor, and analysis of both, based on loss-of-function and gain-of-function experiments, both in vivo and in vitro. This standard of analysis is only now starting to be applied and should help determine whether, within each of the four categories of guidance cues, there are any qualitative differences in the types of guidance events mediated by the different families of effectors (*8*). For instance, are the chemorepulsions mediated by netrins and by semaphorins different in any significant ways? Third, with the identification of guidance receptors, a major thrust will be to determine how guidance signals are transduced and translated into changes in motility and steering of the growth cone (*120*). This task is being facilitated by the discovery of evolutionarily conserved guidance systems, as complementary insights are likely to be gleaned from genetic analysis in invertebrates and biochemical analysis in vertebrates. One byproduct of such studies is likely to be an understanding of how the growth cone integrates the effects of the different cues, attractive and repulsive, that it encounters at any one time, and then translates this information into directed migration. It is possible that the panoply of extracellular signals mediating axon guidance operates through a small number of common transduction mechanisms. Understanding this signal transduction may thus in turn help illuminate the logic underlying the use of particular combinations of guidance molecules to direct specific guidance events. Elucidating this logic remains a central goal of molecular studies of axon guidance.

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1. As reviewed by C. S. Goodman and C. J. Shatz [*Cell* **72**, 77 (1993)], the formation of precise patterns of neuronal connections during development appears to involve the sequential operation of two broad sets of mechanisms: those that require electrical activity in neurons (activity-dependent) and those that do not (activity-independent). The events of growth cone guidance and target recognition described here rely on molecular mechanisms that are apparently activity-independent and that result in an intial pattern of projections that is largely accurate, with the exception that at the target some axons can make a set of connections with target cells that is more diffuse than is appropriate. This pattern of connections subsequently becomes more refined and highly tuned under the influence of the precise patterns of electrical activity in the neurons, as discussed in the accompanying review by L. C. Katz and C. J. Shatz [*Science* **274**, 1133 (1996)].
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Synaptic Activity and the Construction of Cortical Circuits

L. C. Katz* and C. J. Shatz

Vision is critical for the functional and structural maturation of connections in the mammalian visual system. Visual experience, however, is a subset of a more general requirement for neural activity in transforming immature circuits into the organized connections that subserve adult brain function. Early in development, internally generated spontaneous activity sculpts circuits on the basis of the brain's "best guess" at the initial configuration of connections necessary for function and survival. With maturation of the sense organs, the developing brain relies less on spontaneous activity and increasingly on sensory experience. The sequential combination of spontaneously generated and experience-dependent neural activity endows the brain with an ongoing ability to accommodate to dynamically changing inputs during development and throughout life.

The mammalian central nervous system relies on precise synaptic circuits to function correctly. These circuits are assembled during development by the formation of synaptic connections between hundreds of thousands of neurons. Although molecular interactions direct the early formation of circuitry (*1*, *2*), this initial patterning is followed by a prolonged period during which massive numbers of new synapses are added. In this review, we consider how neuronal activity, by guiding synapse formation, elimination, and rearrangements, establishes adult patterns of connectivity and function. We argue that sensory experience, which historically has been viewed as the strongest force guiding circuit formation, is actually a special case of a more general role for neural activity, much of which can be generated spontaneously. We then examine possible mechanisms by which patterns of activity—either spontaneous or evoked by sensory experience—can be translated into patterns of synaptic connections.

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Sensory Experience and Circuit Formation in the Visual System

The role of sensory experience in the formation of neural circuits has been most thoroughly studied in the mammalian visual system. Most current concepts are based on the development of ocular dominance columns in the visual cortex. In carnivores and primates, thalamic inputs to the cortex arising from the lateral geniculate nucleus (LGN) segregate by eye within cortical layer 4 into a series of alternating stripes. These eye-specific stripes form the structural basis for the functionally defined system of ocular dominance columns that span all cortical layers. Early in development, ocular dominance stripes in layer 4 are absent (*3–5*). The LGN axons representing each eye are sparse and simple and overlap within layer 4. By the addition of large numbers of branches and synapses within the appropriate regions and elimination of the sparse collaterals initially present within inappropriate regions, LGN axon arbors gradually form dense, eye-specific patches (Fig. 1) (*6*). These anatomical rearrangements of the presynaptic axons are accompanied functionally by a corresponding change in the synaptic physiology of layer 4 neurons (*7*), the majority of which are initially activated by stimuli presented to either eye but finally come to respond to visual stimulation through one eye only.

The classic experiments of Hubel and Wiesel demonstrated the important role of visual experience in determining the organization of ocular dominance columns (*8*, *9*). If one eye is deprived, even temporarily, of vision by eyelid closure for several weeks in neonatal life, then most of the mature visual cortical neurons are responsive only to stimuli presented to the eye that remained open. Within layer 4, early eye closure greatly enlarges the patches of input from LGN axons representing the open eye, whereas those representing the closed eye are relegated to very small regions (*9*, *10*).

Local cortical circuits undergo similar anatomical rearrangements under the influence of sensory input. In cats, eye closure between 6 months and 1 year of age produces physiological shifts in the cortex's ocular dominance profile, but no anatomical change in the organization of LGN axon terminals (*11*, *12*). This implies that local connections—perhaps those between layer 4 and layer 2/3—remain plastic considerably longer than the longer range connections from the thalamus. In addition, local horizontal connections of pyramidal neurons in cortical layers 2 and 3, which in the adult cortex form periodic clusters of branches that link columns of similar orientation preference, can be altered in response to visual input [reviewed in (*13*)]: Prolonged visual deprivation results in the formation of large, poorly organized clusters (*14*). The clustering of horizontal connections can be altered by inducing strabismus, which prevents cortical neurons from receiving simultaneous inputs