Primer

Axon guidance

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The nervous system has the job of controlling and monitoring events throughout the whole body. But, unlike endocrine cells, which control their targets through long-range chemical cues, neurons communicate through direct cellular contacts, or synapses. As the information content of the electrical signals used by neurons is limited, much of the meaning in neural communication is conveyed by the specificity of the connections between the origin of the signal and its recipient. A failure to make or maintain the appropriate connections can therefore alter the interpretation of neural signals. Thus, precise connectivity is one of the most important issues in constructing a functional neural architecture.

The establishment of correct neural connections begins with a neuron at some distance from its target. Neural connections are formed by means of specialized cellular processes: axons that send signals, and dendrites that receive them. Most neurons send out only one axon. In order to reach the correct target, this axon must be headed in the right direction. Because many neurons are far from their targets, the navigation of an axon to the correct destination often requires a series of decisions that will bring the axon closer and closer to its goal. The process that determines each correct decision is called axon guidance. Despite the immense complexity of the nervous system, axon guidance choices are achieved with remarkable accuracy *in vivo*.

Engine of discovery

The formation of an axon is an impressive feat of cellular morphogenesis. Some neurons extend their axons thousands of cell diameters across a complex cellular landscape. The machinery that lays down the axon is located at the growing tip of the nascent axon, or neurite, within a lively structure called the growth cone. The growth cone is an engine of discovery. It is equipped with an array of fine antennae-like projections (filopodia) with sheets of membrane between them (lamellipodia), that explore the road ahead. The growth cone must interpret extracellular signals, implement directional cell motility, and then construct a stable axonal structure in its wake (see Figure 1a).

The meta-stable architecture of the growth cone depends on cytoskeletal elements of different kinds. A highly dynamic actin network mounts the first response to the environment; actin is assembled at the leading edge and is then drawn back towards the center of the growth cone, providing tension with the outside world through an actomyosin-based mechanism (see Figure 1b). Behind the actin lies a microtubule array that also responds to directional information by reinforcing the part of the leading edge that is heading in the right direction. The organization of these cytoskeletal elements is controlled by a host of associated proteins (such as, profilin and filamin) that regulate the assembly, movement and stability of actin and tubulin polymers as well as the higher-order structure of polymer networks.

Growth cones need an adhesive substrate to move forward. This permissive footing is provided by the surfaces of surrounding cells, or by extracellular matrix proteins that interact with cell and substrate adhesion molecules (CAMs and SAMs) on the growth cone membrane. In addition to providing a means to hold on to the outside world, CAMs and SAMs activate intracellular signals that keep the engine running and the clutch engaged. Although some adhesion molecules have instructive roles in guidance decisions, current evidence suggests that much directional information comes from other classes of molecule (netrins, semaphorins, slits and many others). Such directional factors have two conspicuous effects: attraction or repulsion. These two primal forces can be delivered across long distances to specify a directional vector, or at short distance to determine specific cell contacts.

Getting started

The axon's history begins at the cell body, where growth cones first form. *In vivo*, even this first nucleation event sends the axonal growth cone in the appropriate direction, suggesting external control. In fact, recent evidence indicates that this initial neuronal cell polarity is governed by diffusible factors provided by the surrounding environment. In the developing mammalian cortex and in the leg of the grasshopper, repellent semaphorins are laid out in gradients that define not only the initial neuronal polarity, but also the maintained direction of growth cone motility away from the source of repellent. Like many other examples of cell polarity, the key events in this process include localized cytoskeletal remodeling; in this case, actin destabilization is a common theme.

After the primary growth cone is born it will lay down the main axon shaft. Although this primary growth cone does the first work to define an appropriate path, many neurons connect to final targets via axon collaterals that sprout later from the main shaft. Less is known about the formation and guidance of secondary axonal branches but secreted factors that regulate branching, such as the slits and neurotrophins, also have potent effects on the primary growth cone. These observations suggest that growth cone formation (both primary and secondary) and guidance share a common theme: cell polarity events established by means of discontinuities in cytoskeletal dynamics across the perimeter of the

cell body, axon or growth cone. In many cases these discontinuities are induced by asymmetrically distributed extracellular factors. Asymmetry can be created with a gentle gradient or a discrete source of a factor that either promotes net cytoskeletal assembly or inhibits it (see Figure 1c). Because the overall stability of cytoskeletal elements involves assembly, retrograde transport and recycling of components, there are many ways to generate local differences behind the leading edge.

Decisions, decisions

As in any navigational system, growth cones chart their course relative to landmarks in the environment. The first growth cones to explore the embryonic wilderness, called pioneers, must rely on surrounding cells for their initial cues. Later growth cones have the luxury of using other axons as tracks to follow (a process called fasciculation). In either case, the trajectory to a final target is often complex, involving a series of different landmarks.

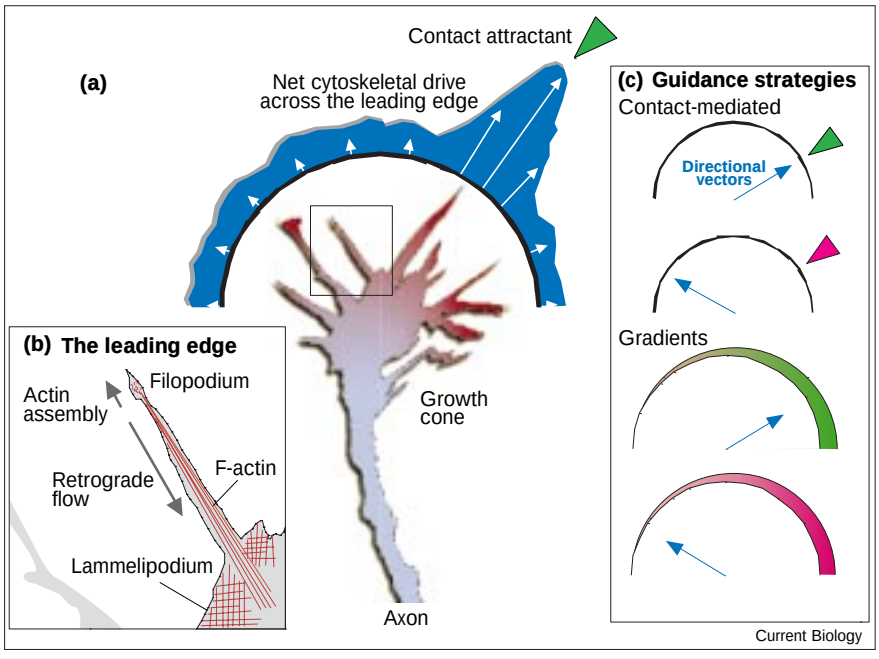
Perhaps the most conspicuous guidance landmarks are cells that act as transient targets for intimate growth cone contact (known as 'intermediate targets'). In vertebrate embryos, intermediate targets are formed by groups of specialized cells, such as the floorplate of the developing spinal cord, which attracts commissural axons that must cross from one side of the body to the other, or the subplate neurons that lie beneath the developing cortex, where thalamic growth cones stop before penetrating the cortical layers. In less complex invertebrate organisms, intermediate targets are often single neuronal, glial or mesodermal cells, such as the 'guidepost' neurons of the grasshopper limb that serve as stepping stones for sensory growth cones.

Roughly speaking, there are two strategies for delivering guidance information: long-range and short-range. Across long distances (millimeters), a common yet elegant theme is the gradient. Gradients can be set up from a point-source of a diffusible factor (as in the case of the chemo-attractive protein netrin, supplied by floorplate cells), or as a gradient in the expression of a localized factor across a continuous array of cells (as in the case of the membrane-bound chemorepellent ephrins found in the targets of retinal ganglion cell axons). Whether pushing or pulling the growth cone, a gradient is an economical way of defining a general direction between points along the path.

At close range, growth-cone interactions can be controlled with greater precision using discrete boundaries of contact-limited guidance cues. These molecules include the many CAMs that control the selective fasciculation of specific follower growth cones within a maze of other axons, such as fasciclin II and N-cadherin. In addition, there are other classes of cell-surface ligand expressed along the path or by target cells (for example, semaphorin I). In fact, axon guidance *in vivo* results from a symphony of different signals, often converging on a particular location (see green box). Recent studies in model organisms (flies and worms) confirm that growth cones simultaneously integrate different types of guidance cue to reach decisions. This combinatorial approach vastly increases the information content available from a limited number of signal molecules.

Transitions from one guidance landmark to the next present choice points where growth cones must perceive old and new information, and express a preferential appetite for the next set of cues. This raises an important question: how does a

Figure 1



The directional motility of the growth cone (a) is largely dependent on the dynamic cytoskeletal structures that underlie the filopodia and lamellipodia at the leading edge (b), where actin (red) assembly occurs. Microtubules are not shown. Axon guidance factors have two main effects on growth cone movement, attraction (green) and repulsion (magenta); these activities (c) can be delivered at short range (contact-mediated) or long range (gradients). Many such factors are thought to steer the growth cone by inducing asymmetry in the net cytoskeletal assembly (or 'drive') across the leading edge (a).

Axon guidance molecules

Some of the major classes of molecule that influence axon guidance behavior. Although complete binding relationships are far more complex than indicated here, pairs of ligands and corresponding receptors are listed on the same horizontal line.

Ligand class	Ligand type	Receptor class
Extracellular matrix	Laminin	Integrins (subrate adhesion molecules)
	Fibronectin	
Cell adhesion molecules (CAMs)	Immunoglobulin superfamily	Immunoglobulin-CAMs (and fibroblast growth factor receptor tyrosine kinase)
	Cadherin	Cadherins (and fibroblast growth factor receptor tyrosine kinase)
	Leucine-rich repeat	Leucine-rich repeat-CAMs
Semaphorins	Secreted	Neuropilins
	transmembrane	Plexins
Slits		Roundabouts
Netrins		DCC/unc-40 and unc-5
Ephrins		Eph receptor tyrosine kinases

growth cone stop wanting the old cue(s)? One strategy is to have a set hierarchy where each cue on the sequential menu is more delicious than the last. Another approach is to actively disengage one input to allow a growth cone to take interest in the next, as seen in the de-fasciculation of axon contacts by means of either N-CAM glycosylation or secreted factors such as 'beaten path'. More recent work on the signaling machinery downstream of guidance factors suggests that growth cone response is highly dynamic and affected by modulators that may have an important part in shaping navigational behavior *in vivo*.

A mind of its own

In order to interpret guidance information and make correct decisions in a time-scale of minutes the growth cone needs a mind of its own, independent of machinery located in the distant cell body. Axon guidance receptors, like other types of receptor, seem to collaborate with a variety of intracellular proteins to communicate with their effector systems. This is true for both CAMs and SAMs that must find anchorage beneath the cell membrane, and receptors for chemotactic cues that turn the growth cone. Some signaling pathways are controlled by protein phosphorylation, through a dynamic antagonism of kinases and phosphatases that is well suited for a changing environment where the growth cone must be poised for new directives. Other components may function by recruitment to the cytoplasmic domains of receptors at the membrane cortex, such as the guanine nucleotide exchange factors that locally activate GTPases from the Rho family, or adaptor proteins that bind and assemble multiple signaling partners. Although few axon guidance signaling pathways have been traced from cell surface to cytoskeleton (or to other effectors), it is likely that many of the downstream components are shared between different types of receptor, providing opportunities for the integration of different simultaneous inputs.

Surprisingly, most secreted guidance factors are capable of eliciting both attractive and repellent responses, depending on the recipient. The polarity of the response will often be determined by the receptor type, as the receptor cytoplasmic domain determines which intracellular signaling molecules will be activated by a ligand. But it is also possible to invert the response of a given receptor by means of intracellular modulators such as cyclic nucleotides (cAMP or cGMP). This points to signal integration and the importance of context. Although it is likely that guidance information is integrated at different levels in the signaling hierarchy, no one really knows exactly how this works.

The end

For accurate navigation to targets, the growth cone needs a gas pedal and a steering wheel but it also needs brakes. Somehow the appropriate target must send a 'stop' signal to allow stable contacts (synapses) to be initiated. Like pathway selection, target recognition is likely to involve combinations of signals.

Different types of contact-limited cue (for example, leucine-rich repeat proteins, immunoglobulin-CAMs and ephrins) clearly contribute to target recognition; however, the nature of the transition from motile growth cone to pre-synaptic terminal is largely a mystery. Of course, synaptic endings must also be remodeled over time for the neural network to acquire new properties. Although some axon guidance and/or branching factors might also control this plasticity, we're only just beginning to understand the end.

Key references

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