The growth cone: an integrator of unique cues into refined axon guidance
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Abstract
One of the challenges to understanding nervous system development is to establish how a fairly limited number of axon guidance cues can set up the patterning of very complex nervous systems. Most of the recent insights relevant to guidance mechanisms have come from cell biologists focusing on processes and molecular machinery controlling the guidance responses in the growth cone.

Introduction and context
The axons of many neurons migrate along both the dorsal-ventral and the anterior-posterior axes at different phases of development, and may also cross the midline. An individual axon may be subjected to opposing attractive and repulsive forces coming from opposite sides in the elongation axis or perpendicular to it (reviewed in [1–3]). Pathfinding events have also been shown to be dependent on the normal pattern of bursting electrical activity [4]. However, even if the list of candidate guidance cues (which includes diffusible and bound guidance molecules) continues to increase [5], it could be considered that genetics and biochemistry have allowed the identification of the key molecules and provided an important insight into their function. It is now clear that complex trajectories can be generated with very few guidance cues, as illustrated by the multiple divergent pathways specified by a single cue. Generally, transcriptional regulation specifies the path (reviewed in [6]), whereas complex post-transcriptional and post-translational regulations control growth along that path. Importantly, molecules that were first discovered as ‘developmental cues’ are now emerging as important factors in neurological diseases and injury in adults (reviewed in [7]).

These guidance signals share common functional properties leading to actin cytoskeleton remodelling. Indeed, the direct or indirect interaction between the receptors of these guidance cues and actin modulators is the final step of the signalling cascade constituting the fundamental mechanism defining the orientation and extension of the axonal growth cone. However, how a growth cone samples and integrates various signals both in space and in time, and subsequently coordinates the dynamics of its membrane, cytoskeleton, and adhesion to generate specific responses, is still unknown. To tackle this question, the tools of genetics and biochemistry need to be coupled with tools from other fields, and most of the recent insights relevant to guidance mechanisms have come from cell biologists.

Major recent advances
It has become increasingly clear that a neuron can switch its response to a given cue over time, although only fragmented data are available to explain how the growth cone adapts its response dynamically, depending of the source and distribution of the guidance cue all along its pathway. Undeniably, the recent reports point to the intricate diversity of the mechanisms at play.
**Combinatorial interactions and dynamics of receptor expression at the cell surface**

First, because extracellular cues and membrane receptors constitute the first signalling modalities, selectivity in ligand-receptor binding is likely to dictate the outcome of the response via downstream signalling events. In this context, the combinatorial physical interactions that a receptor/binding subunit might undergo with other surface molecules is of major importance and can in some instances explain bifunctionality. Semaphorin 3E can mediate both attraction or repulsion, depending on whether neuropilin-1 is part of the receptor complex [8]. Additional illustrations on how diversity at the cell surface specifies different axon responses are given by splice variants of Slit [9] or by the alternative splicing of the Down syndrome cell adhesion molecule (Dscam) gene that potentially generates 38,000 closely related transmembrane proteins of the immunoglobulin superfamily. Experiments reducing the entire repertoire of Dscam ectodomains to just a single isoform allowed for the proposition that Dscam diversity provides each neuron with a unique identity by which it can distinguish its own processes from those of other neurons [10]. Moreover, netrin-1, already well known to repulse growth cones when it binds to a UNC5H family member and to attract growth cones when it binds to the receptor deleted in colorectal cancer (DCC), can also bind to Dscam to mediate outgrowth and turning [11], while Dscam in turn can act as a receptor for other ligands that have not yet been identified [12].

Second, not only the repertoire of interactions but also the number of receptors exposed by a growth cone might change dynamically. Controlled endocytosis appears to be a major factor in receptor trafficking. An example of such a regulatory mechanism is the response of a neuron to netrin-1, which regulates the levels of cell surface UNC5A receptor via netrin-1-independent A2bR (adenosin receptor) signalling [13,14].

Third, precise spatial distribution/asymmetry of guidance receptors at the growth cone also appears to control the axon response. The means by which the asymmetry is established and maintained are unclear as asymmetric sensitivity could result from, and in turn trigger, several mechanisms. Powerful techniques of detection allowed for the proposition that binding of a guidance cue to its receptor causes its asymmetric localization on the surface. For example, a gamma-aminobutyric acid (GABA) gradient creates an asymmetric redistribution of GABA receptors [15] which might result in an asymmetric elevation of the Ca\(^{2+}\) concentration in the growth cone. The receptors symmetrically expressed at the leading edge could also be redistributed by interaction with the cytoskeleton [16], by their recruitment into microdomains [17], or by interaction with the Rho GTPases [18].

**Intracellular events**

Besides events occurring on the surface, several issues concerning intracellular events have recently received attention. First, an early membrane-associated event responsible for signal transduction controlling growth cone dynamics appears to be the relative levels of cyclic nucleotides [19] and Ca\(^{2+}\) concentration [20]. Of note is that an asymmetric relocalization of the guidance receptor should lead to asymmetric activation of the downstream signalling pathway. Asymmetric elevation of the Ca\(^{2+}\) concentration can mediate both attractive and repulsive axon guidance. Calcium signalling via calcium-induced calcium release (CICR) promotes an attractive turning response, whereas Ca\(^{2+}\) signals that are not accompanied by CICR trigger repulsion. Interestingly, growth cone attraction, but not growth cone repulsion, involves asymmetric vesicle transport along pre-existing microtubules possibly regulated via protein phosphorylation downstream of CICR, followed by a local burst of exocytosis [21]. It is not yet known whether growth cone repulsion is due to local endocytosis which would be triggered by the repulsive Ca\(^{2+}\) signals. Second, growth cone attraction as well as repulsion also depends on asymmetric cytoskeletal dynamics which eventually provides the mechanistic force for directional movement. Small GTPases of the Rho family are important for regulating the actin cytoskeleton [22]. Recent results have bridged a gap in the understanding of the signal transduction pathway of the prototypical attractant netrin, showing that signalling activation of Rac proteins via the activation of guanine nucleotide exchange factor (GEF) [23,24] locally recruits MIG-10 (the Caenorhabditis elegans ortholog of lamellipodin), which leads to actin polymerization and results in asymmetric formation of lamellipodia and filopodia [25]. Recent studies also suggest that the planar cell polarity pathways defined by their ability to direct the development of obviously polarized cellular architectures can mediate changes in the cytoskeleton fundamental to axon guidance (reviewed in [26]) and intersect with the Par complex [27].

**Local protein synthesis**

The capacity of growth cones to locally translate proteins in response to guidance cues, including netrin-1, slit-2, and semaphorin 3A, is now well established. An emerging model, termed the ‘differential translation’ model, proposes that attractive and repulsive cues induce asymmetrical translation of proteins that build up or break down the cytoskeleton respectively (reviewed in...
[28]). It is possible that local translation permits regulation distinct from regulation achieved by identified canonical pathways known to mediate cytoskeletal remodelling in axons. Although several studies point to the importance of the microRNA pathway in the regulation of axonal translation [29], the detection of translation events with spatial and temporal precision is an emerging challenge.

Future directions
Many studies are now providing insights into the transduction and integration of signals in the growth cone. However, a full understanding of growth cone behavior still eludes us and remains a long-term goal. Progress might come from different directions. Given the considerable complexity of the underlying molecular interactions, it seems probable that systems biology approaches are required to further improve our understanding. Although challenging due to the small amount of material obtainable from axons, developing screens to uncover whole populations of proteins translated in response to specific guidance cues might help to uncover functionally coherent groups. In parallel, analogies can be made with other systems such as chemotaxis in neutrophils or in Dictyostelium, since there is a similarity in several of the molecules involved [30].

On the other hand, because of the huge number of molecules already known to have direct and indirect growth-control capabilities, perhaps it is not particularly relevant to determine what types of molecules are required for the actual occurrence of axon guidance, but rather it is the mechanisms by which they accumulate at a given place in space that are the most interesting to decipher. This concept applies to a large number of signalling processes. Sensitive methods are now available to quantify the localization of molecules within different cellular compartments, to detect subtle changes, or to define their threshold levels in a certain location that are necessary to trigger subsequent events. These approaches rely on cutting-edge imaging technologies (for example [31]) that shall be instrumental in opening a window into the intricate biochemical activities that occur inside the growth cones.

Abbreviations
CICR, calcium-induced calcium release; DCC, deleted in colorectal cancer; Dscam, Down syndrome cell adhesion molecule; GABA, gamma-aminobutyric acid; GEF, guanine nucleotide exchange factor.

Competing interests
The authors declare that they have no competing interests.

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References

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