Axonal Pathfinding

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Introduction

Building a brain requires the precise formation of connections between vast numbers of neurons, often separated by significant distances. Axon pathfinding is thus a crucial process in the development of a functioning nervous system. A better understanding of the principles and mechanisms underlying axon pathfinding has both clinical and broader practical importance. From a clinical perspective, our ability to treat or prevent some neurological defects will be improved by a better understanding of how axon guidance can fail. Furthermore, the regeneration of damaged nerves requires axons to reconnect to their appropriate targets; hence, understanding axon guidance will be necessary for the development of therapeutic techniques. In a broader context, our understanding of axon pathfinding ties into our understanding of the nervous system as a whole: what principles underlie its formation and function? can we harness those principles in order to improve our own engineering processes, such as in the construction of self-wiring computers?

Mathematical and computational models are very useful tools for understanding the constraints on nervous system development. Ultimately, such constraints are quantitative and set by the physics of the system; hence, they must be modeled mathematically to yield the best predictive power and generate strong hypotheses. Models may also prove valuable in the development of therapies. For instance, how can axons be made to grow toward a specific target area? What additional information is needed by axons which are failing to develop correctly? Sufficiently detailed theoretical models have the potential to guide experimental research in axon pathfinding through simulations done *in silico*.

Experimental Data

Guidance Cues

Axons grow along their correct trajectories by following a molecular map consisting of spatiotemporal patterns of guidance cue molecules. Four main families of molecules have been identified based on their guidance abilities – the netrins, the Slits, the semaphorins, and the ephrins – consisting of approximately 100 distinct molecules altogether (although several other classes of molecules also provide guidance information for axons, including the neurotrophins and some classical morphogens). The netrins were first identified as attractive guidance cues which direct contralaterally projecting neurons toward the midline. The transmembrane proteins DCC and Unc-5 have been shown to act as receptors for netrin-1. Both netrins and their receptors are highly conserved between species. Netrin-1 is known to have a bifunctional role, typically attracting growth cones expressing only the DCC receptor but repelling growth cones expressing both DCC and Unc-5. The Slits and their receptors, the roundabout family (the Robos), were first identified as repellents preventing contralaterally projecting neurons from recrossing the midline. Subsequently, they were also shown to stimulate axon outgrowth and branching. The semaphorins appear to act primarily as short-range cues which repel axons from particular regions or, by forming the walls of corridors, hem axons into a preferred path. However, they have also been reported to act as long-range chemoattractants. Semaphorins are classified by their structure into eight groups. They signal through multimeric receptor complexes, with the precise structure of a complex determining its specificity for a semaphorin subgroup. Semaphorin receptor molecules include the neuropilins, the plexins, and the cell adhesion molecule L1. The ephrins are substrate-bound molecules best known for their role in the formation of topographic maps in the central nervous system. For example, the graded expression of the Eph tyrosine kinases – the ephrin receptors – in the retina combined with graded expression of the ephrins in the tectum aid in the formation of an ordered topographic mapping between the two structures. Similar strategies appear to orchestrate the formation of other topographic maps. Although ephrin/Eph signaling induces axon repulsion in these examples, under other contexts the ephrins can also act to attract axons.

The Growth Cone

Growing axons are tipped by special sensorimotor structures known as growth cones. These probe their local environment and, depending on the signals they detect, direct axon outgrowth, turning, branching, and pruning. Growth cones exhibit complex morphology, as illustrated in Figure 1. They are conceptually divided into three sections: an actin-rich peripheral region, a transitional region, and a central region containing organelles and microtubules. Fingerlike protuberances extending from the edge of the growth cone known as filopodia are supported by bundles of filamentous actin (F-actin). These appear to act as sensory devices, extending the effective

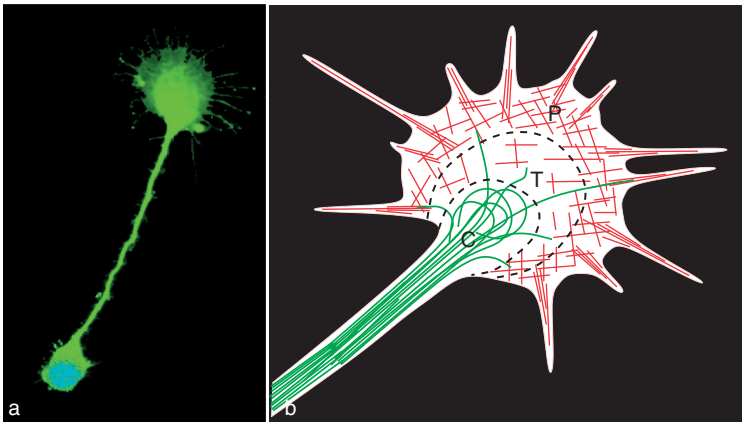

Figure 1 (a) Rat superior cervical ganglion neuron *in vitro*. The neuron was grown on a substrate of laminin for 48 h and then fixed and stained for β-tubulin. The cell body is at the bottom left, and the growth cone is at the top right. (b) Anatomy of the growth cone. This figure illustrates the division of the growth cone into three domains: transitional (T), peripheral (P), and central (C). The peripheral domain is rich in actin, which exhibits two forms of organization: loosely linked networks giving rise to lamellipodial structures and tight bundles supporting filopodia. The central domain contains organelles and microtubules which extend from the axon shaft into the growth cone. Rat SCG neuron image courtesy of Z Pujic.

sensing range of the growth cone. Structures based on more chaotic meshworks of F-actin are known as lamellipodia and have also been implicated in growth cone movement.

Growth cones undergo constant morphological change thought to be driven by remodeling of the actin network controlled by actin-binding proteins such as myosin, ARP2/3, and WASP. Growth cone motility appears to be driven by an 'actin treadmill.' Unpolymerized G-actin diffuses outward into the peripheral region and preferentially polymerizes near the cell membrane. The entire network of polymerized actin is drawn toward the central region by myosin and undergoes depolymerization in the transitional region. This constant cycle of polymerization, retrograde flow, and depolymerization is thought to generate traction when coupled to a permissive substrate through adhesion molecules.

Microtubules also play a significant, although unclear, role in growth cone motility and axon guidance. Interactions between microtubules and actin in the transitional region appear to have a strong influence on axon outgrowth and guidance.

Guidance cues influence growth cone behavior, and subsequent axon outgrowth, through cytoskeletal effectors activated or inhibited by cascades of intracellular second messengers triggered by receptor binding. Several molecules have been implicated as playing roles in this process, particularly calcium and the cyclic nucleotides cAMP and cGMP. These have been the focus of much attention, with the finding that, in some circumstances, changing the relative concentrations of these molecules within the growth cone can switch the effect of several guidance cues from attraction to repulsion or vice versa. Further intriguing findings have demonstrated that protein synthesis occurring locally within the growth cone is necessary for correct growth cone behavior.

Theoretical and Computational Models of Axon Pathfinding

Axon Extension and Branching

Experimental evidence suggests that tubulin molecules are synthesized only in the soma and then assembled into microtubules predominantly in the growth cone. This implies that axon outgrowth is limited by the rate at which microtubules can be transported to regions of active extension. A number of theoretical models have explored this idea, including various effects such as diffusive and active transport of tubulin monomers, competition between neurites for tubulin, viscoelastic stretching of axon segments, calcium-induced microtubule depolymerization, and varying intrinsic rates of tubulin polymerization and depolymerization within different growth cones. These models have been successively refined, ultimately incorporating compartment-based modeling with dynamic compartment allocation. Most strikingly, this modeling program has demonstrated that small variations in polymerization and depolymerization rates in the growth cones of different neurites can lead to sharp changes in elongation rate, including growth cone pausing and neurite retraction.

Growth cone behavior and axon extension are thought to be mediated by partially independent but related processes. This has led to modeling work

focused on characterizing the interaction between the two. The majority of this work has been phenomenological, using sophisticated mathematical machinery to better describe experimental data. Work in the mid-1980s showed that in some circumstances, axon elongation can be regarded as a one-dimensional random walk, with parameters varying with neuronal type, substrate, and chemical environment. In the mid-1990s, this was extended by performing correlation analysis which showed that axon outgrowth dynamics exhibit significant anticorrelation. Subsequent analysis showed significant correlation between the dynamics of microtubule polymerization in the central region and growth cone advance.

Growth Cone Morphology

The complex and dynamic nature of growth cone morphology further complicates our understanding of the contribution of growth cones to axon pathfinding. By statistically analyzing time-lapse images of growth cones undergoing dynamic changes in morphology, researchers have developed probabilistic rules specifying the likelihood of filopodial initiation and retraction, and also the spatial distribution of filopodia in terms of a limited number of parameters. In this model, growth cone morphology is described by the instantaneous length and angle of each filopodium, and the dynamics of the filopodia are characterized by the following parameters: the rate at which filopodia extend, the rate of retraction, the average rate at which new filopodia are initiated (modeled as a Poisson process), and the shape parameters for a gamma distribution that gives the time over which a filopodium extends before retracting. The model also specifies a simple conditionally random rule for where a filopodium initiates, and it assumes that filopodia extend radially from the center of the growth cone. Computer simulation then gives qualitatively realistic morphologies, which also satisfy quantitative constraints such as the correct average number of filopodia. By mapping the effects of external cues onto the parameters of the model, one can hope to gain some intuition as to how those cues might operate.

Other researchers have directly modeled the processes underlying actin dynamics in order to understand filopodial formation, stability, and behavior. This analysis indicates that the maximum length of a filopodium is determined by the number of bundled actin filaments in its core. For less than approximately 10 bundled actin filaments, the strain exerted on the bundle by the membrane is sufficient to cause buckling for even very short filopodia. As the number of included filaments increases, it becomes less likely that a filopodium will buckle; however, more G-actin is required for the structure to continue extending. Thus, when the number of filaments is too large, a filopodium is also unable to extend. The best tradeoff between stability and G-actin depletion is achieved with approximately 30 actin filaments. The model predicts average filopodia lengths between 1 and 10 μm, which are in agreement with experimental data. Similar work has examined the mechanisms behind lamellipodial structures.

Axon Turning and Guidance

Axons are thought to be guided by external cues through two processes: gradient-based guidance, in which the growth cone attempts to climb or descend a concentration gradient (chemoattraction or chemorepulsion), and contact-mediated guidance, in which the growth cone interacts with small regions of highly concentrated guidance cues and modulates its behavior accordingly. For gradient-based guidance, the growth cone must detect a potentially shallow gradient in the presence of noise, whereas for contact-mediated guidance the growth cone is essentially involved in a search process.

Gradient guidance Single cell chemotaxis – the attraction or repulsion of organisms such as bacteria, leukocytes, or slime molds by chemical gradients – has received a large amount of theoretical attention. Much of this can be directly applied to the case of the growth cone. Of particular relevance is a seminal contribution by Berg and Purcell, who argued that gradient detection by any small sensing device is fundamentally limited by statistical fluctuations, both due to variations in local ligand concentration and due to the inherent stochasticity of receptor binding. Growth cones are believed to sense and respond to gradients by comparing receptor binding across their spatial extent: the side of a growth cone exposed to the highest concentration of ligand will, on average, also display the largest amount of receptor binding. If growth cones do use such a spatial-sensing strategy, then in order for a growth cone to detect and reliably respond to a chemical gradient, the noise due to fluctuations in receptor binding cannot be much larger than the difference in receptor binding across its spatial extent. By modeling the physics of receptor–ligand interaction, one can estimate the limitations growth cones face when responding to chemical gradients. If the root mean squared error in a concentration measurement is given by σ_C, then the error associated with taking the difference between two such measurements is $\sigma_{\Delta C} = \sqrt{2\sigma_C}$. This gives an order of magnitude lower bound on the difference in concentration, ΔC, that the growth cone can

detect: $\Delta C_{min} \approx \sigma_{\Delta C}$. Using a simple model of receptor binding mechanics, it has been shown that for the timescale on which growth cone behavior usually occurs ($\sim$100 s), growth cones can detect gradients with a steepness of between 1% and 10%, depending on whether the guidance cue is diffusing freely or is substrate bound. However, over much longer periods (several days), experimental work has demonstrated that growth cones can respond to gradients of 0.2% or less across their spatial extent. Furthermore, at sufficiently high and low concentrations, almost all or almost no receptors are bound: this leads to further reductions in sensitivity. Experimental and theoretical work has confirmed that the highest sensitivity is achieved when approximately half of the receptors are bound, which occurs when the concentration is equal to the dissociation constant for binding.

Aside from these general constraints on gradient detection, models have also been developed which directly simulate the behavior of growth cones and axons in the presence of guidance cue gradients. Several models have focused on biochemical networks which are putatively responsible for growth cone motility and guidance. Such models are difficult to construct, partly due to the complexity of growth cone biochemistry and partly due to the lack of experimental data on important quantities such as reaction rate constants, concentrations, and interactions between molecular species. One model has focused on the Rho-GTPase signaling network, which is known to play an important role in actin-driven cell motility. Due to incomplete experimental data, the investigators took a qualitative approach, simulating the behavior of several plausible interaction networks and kinetic constants and using the results of these simulations to form hypotheses about the underlying mechanisms of growth cone motility. They found that the Rho-GTPase network undergoes a sharp transition in its dynamics when a threshold concentration of a particular signaling molecule is reached. The authors linked these two dynamic behaviors to different modes of growth cone motility, developing a model which could reproduce some experimentally observed phenomena.

Other models have placed less emphasis on specific biochemical mechanisms and have focused instead on the potential role of filopodia in axon guidance or on more general signal-processing strategies that a growth cone may implement, such as temporally or spatially averaging receptor inputs in order to reduce noise. Spatial averaging involves pooling the inputs from multiple receptors, whereas temporal averaging combines information from different time points. An interesting conclusion from this study is that spatial averaging provides the most benefit when the average is taken over approximately one-third of the growth cone's spatial extent. This optimum averaging range occurs because although spatial averaging reduces noise in a local concentration measurement, it also reduces the spatial resolution of the measurement. Because gradient detection requires concentration measurements to be made at multiple locations, the advantages gained in noise reduction are offset by the loss in resolution. Assuming growth cones use such a strategy, this has implications for the intracellular signaling network, suggesting that second messenger molecules implementing the spatial averaging process must diffuse at a rate much slower than expected for cytosolic compounds. One possibility is that spatial averaging is achieved through membrane-bound molecules.

In addition to detecting a chemical gradient, the growth cone must also amplify the possibly extremely shallow gradient in receptor binding in order to achieve a definite motile response. Understanding the mechanisms underlying this amplification has been a general focus for experimental and modeling work on microbial chemotaxis. In one influential model, amplification is achieved by coupling the external signal to a pattern formation system involving local activation and long-range inhibition. The system begins in a spatially symmetric, but unstable, steady state. Symmetry is broken by the external signal, which pushes the system into a stable, asymmetric state that reflects the direction in which the symmetry was broken. A difficulty with this approach is that the system then becomes stuck, unable to respond to new inputs such as a change in the external signal. Several additional mechanisms have been proposed to work around this, each postulating a second process which serves to reset the system to its original, unstable state. Further generalizations of this class of models suggest mechanisms for the formation of filopodia and generate testable predictions for the spatiotemporal distribution of such structures.

Contact-mediated guidance Axons are also guided by cues which are more tightly localized in space, referred to as short-range or contact-mediated cues. For example, filopodial contact with single cells expressing appropriate cues can entirely redirect an axon's trajectory. For this kind of guidance, noise is less of an issue because the signal is essentially binary: either the growth cone contacts the cue or it does not. In this situation, an appropriate theoretical framework is that of stochastic search. The question of how filopodial dynamics of the growth cone affects its ability to locate and respond to highly localized guidance cues has been addressed with the aid of the models describing growth cone morphology in terms of filopodial dynamics. The efficacy with which a growth cone is able to locate a guidance cue has been mapped against

the parameters defining the dynamics, suggesting some behaviors one might expect to observe depending on the geometry of the guidance cue distribution. This work suggests that filopodial dynamics are set, and possibly modulated, in order to increase a growth cone's ability to detect and respond to relevant cues. In a more abstract approach, growth cone movements were described by a combination of stochastic (e.g., deflection by random adhesion to the substrate) and deterministic (e.g., a tendency to move in the direction of past axon extension) motions. The growth cone was found to more effectively respond to short-range guidance signals when the two processes contributed equally. This prompted the authors to propose that growth cones modulate the relative influence of stochastic and deterministic movements depending on the importance of short-range cues at different stages in development – a suggestion consistent with experimental observations of growth cone behavior.

Axon–Axon Interactions

The models described so far consider the guidance of single neurons in isolation. However, the development of the nervous system involves the correct guidance of many axons simultaneously, and it is well established that axons use one another as additional sources of information during development. One of the earliest computer models of axon guidance attempted to explain the characteristic 'sheetlike' pattern of axon outgrowth observed in the formation of the ventral commissure of the spinal cord. Using a descriptive model of individual axon behavior, including several experimentally observed features of ventral commissural axon growth – a tendency for straight growth, for initial outgrowth to be directed ventrally, and for growing axons to extend preferentially over the surrounding matrix and not other axons – this work attempted to distinguish the most important features of individual axon behavior for the formation of axon sheets. From computer simulations, the authors concluded that initially polarized outgrowth, a suitably high density of neurons, preferential adhesivity for extension over the substrate rather than other axons, and a tendency for straight growth were sufficient to generate the observed patterns. Another model examined the possibility that growth cones secrete diffusible guidance cues in order to attract or repel one another to create or break up axon bundles.

Topographic Map Formation

A specific example of axon guidance that has been well studied theoretically is the formation of the topographic map between the retina and optic tectum/superior colliculus. In 1963, Roger Sperry first proposed that such maps could arise because gradients of molecular labels in the retina are matched to gradients of labels in the tectum. The subsequent discovery of gradients of Eph receptors and their ligands, the ephrins, in the retina and tectum confirmed this prediction. However, a large number of experiments investigating how such matching might work in detail have suggested that several other constraints are also important. Since the 1970s, numerous theoretical models of such map formation have been proposed in order to gain insight into this complexity. Some of the simplest propose sorting mechanisms, whereby an initial random map is refined by comparing the retinal origin of axons terminating at neighboring sites in the tectum. Others have hypothesized that tectal labels are at least partly induced or modified by transport of retinal labels into the tectum. Several models have highlighted the importance of competition in map formation, both between axons for tectal target space and between tectal targets for axons. Another important theme has been cooperative effects between axons, somewhat similar to the axon–axon interactions discussed previously. Increasing data on the precise role of Eph/ephrins in map formation have provided new challenges for such models, many of which are yet to be addressed.

Guidance Cue Patterning

A further area of active research aims to understand how guidance cue patterns are generated in the first place, and how effectively particular patterns can guide axons. The modeling of gradient systems in developing organisms has a long history, and gradients are thought to be a primary means for generating spatial ordering. In general, molecules expressed as gradients in order to provide spatial information are known as morphogens, and several classical morphogens have been shown to also guide axons.

A number of models have been proposed to explain how gradients of appropriate shape and stability could be set up. The simplest model assumes that the molecule of interest is diffusing away from a continuous source through a homogeneous medium. More complex models recognize the inhomogeneous nature of the medium, degradation of molecules, binding of molecules to cells, endocytosis, and active transport processes. A further complication which arises when attempting to generalize results from one experimental model to another is that of scaling: gradients form on a typical length scale, and different species have embryos of different sizes at developmental stages when axon wiring is forming. Hence, a system which works in one embryo may not work in another.

Additional constraints are placed on the formation of gradients useful for axon guidance. The ability of a

growth cone to detect and respond to a gradient varies with background concentration and gradient slope. The minimum gradient that a growth cone can detect over a particular background concentration specifies limitations on gradient-based guidance. It allows the construction of optimal gradients, in which the gradient slope is always equal to the minimal detectable gradient for the growth cone. Following this line of argument, coupled with estimates of parameters central to the model, it can be shown that the maximum distance over which growth cones can be guided by an optimal gradient is on the order of 1 cm.

Future Directions

This article provided an overview of the kinds of models which have been applied to axon guidance and how these have helped us to understand axon pathfinding. However, research of this kind is still at an early stage. Important questions remain to be answered and are the focus of active research. For instance, how sensitive can growth cones be to gradients of guidance cues? How close do they come to achieving fundamental sensitivity limits? What are the actual mechanisms they use to detect gradients? How do developing neurites integrate information from multiple guidance cues? What searching strategies do growth cones use to locate local guidance cues? What roles do axon branching and pruning play in axon guidance? How do microtubules and the F-actin cytoskeleton interact to support axon outgrowth and steering? How much of a role do axon–axon interactions play in the formation of the nervous system, and when are they important?

In addition to fresh modeling approaches, answering these questions will require significant experimental advances. A wealth of experimental data is available on axon guidance, but most studies have been aimed at identifying guidance cue molecules or intracellular molecules mediating or eliciting particular behaviors. Although such data are obviously crucial, in order to generate sufficiently constrained models, data of a more quantitative nature are needed. Recognizing this need, experimental techniques for producing well-controlled and flexible patterns of guidance cues have been developed. Ultimately, these techniques should allow us to develop better constrained models and, using them, obtain additional power to tease apart the mechanisms and principles underlying axon guidance.

Finally, the discovery that new neurons are constantly being born in adult brains opens up another area for exploration. These nascent neurons must somehow find their way to their appropriate niches and extend axons to make functional connections. Modeling axon guidance thus has a central role to play in understanding both the initial development and the normal functioning of the nervous system.

See also: Axon Guidance: Morphogens as Chemoattractants and Chemorepellants; Axon Guidance: Building Pathways with Molecular Cues in Vertebrate Sensory Systems; Axon Guidance: Guidance Cues and Guidepost Cells; Axonal Regeneration: Role of Growth and Guidance Cues; Cognitive Neuroscience: An Overview; Computational Methods; Growth Cones; Neuroplasticity: Computational Approaches.

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