CMLS, Cell. Mol. Life Sci. 55 (1999) 1355–1357
1420-682X/99/111355-03 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Introduction: invertebrate axons find their way

D. Van Vactor* and L. J. Lorenz

Department of Cell Biology and Program in Neuroscience, Harvard Medical School, Boston (Massachusetts 02115, USA), e-mail: Davie@hms.harvard.edu

Abstract. The mechanisms that generate the immense complexity of synaptic connections within the developing nervous system have fascinated biologists for decades. Analysis of nervous system development in simple systems, such as insects, has made a major contribution to our understanding of the cellular and molecular mechanisms that control the formation of axon pathways and precise connections. This enterprise has a long, interesting, and somewhat controversial history. This collection of reviews on axon guidance in insects provides a brief update to integrate current molecular and developmental insights in a number of areas from initial axon pathfinding to the recognition of synaptic partners.

Key words. Axon; growth cone; guidance; target; recognition; invertebrate; neural development.

Insects and axons

In the developing nervous system, there is a very special and somewhat mysterious relationship between a neuron and its ultimate synaptic partner. Cajal, discoverer of the synapse and the growth cone, referred to synapses as 'protoplasmic kisses . . . the final ecstasy of an epic love story' [1]. At the beginning of this great story, the amorous neuron, often distant from its betrothed, sends out its axon across the embryonic landscape. Along the way to its final destination, the axon encounters cues that influence its pathway selection and send it in the direction of its final target(s). The journey ends with the consummation of that special bond between the neuron and the object of its affection.

The origin of developmental studies in axon guidance is often traced back to the classic work of Cajal and Harrison near the turn of the last century [1]. This seminal work in vivo and in vitro was conducted in different vertebrate systems. However, over the past three decades, many striking discoveries in the field of axon guidance have been made in invertebrate organisms, such as grasshopper and, more recently, *Drosophila*. Interestingly, the history of insect neurobiology has its roots well before the twentieth century [2–4]. In fact, even Cajal can be credited with important contributions to the characterization of the anatomy of the Dipteran visual system [5]. Although such pioneering studies paved the way for later work, the most influential studies of insect axon guidance began in the late 1970s. These developmental studies helped establish a foundation for the molecular insights of the last decade—providing strong evidence that specific cell-cell communication events lie at the heart of the guidance process.

Axon guidance in the intact organism

Perhaps the most influential insect for the developmental neurobiology of the 1970s and early 1980s was the grasshopper. The grasshopper embryo is large and amenable to whole-embryo tissue culture, permitting researchers to explore the rules that govern developmental events within the intact organism. Although early general descriptions of neurogenesis in the grasshopper central nervous system (CNS) were published in the early 1890s [6, 7], detailed characterization of embryonic development did not appear until the mid-1970s [8, 9]. This later work revealed a relatively simple and highly stereotyped array of neuroblasts and neuronal progeny which made it possible to explore the role of cellular interactions in shaping the fate and axon pathfinding decisions of identified cells [see, e.g., ref. 10].

* Corresponding author.

With the advent of axon-tracing dyes to follow the behavior of identified neurons, and single-cell ablation to test the importance of cells or axons along the path, elegant studies established that specific pathfinding decisions require discrete interactions between growth cones and particular cell surfaces. In the periphery, ablation studies of sensory axon guidance in the grasshopper revealed specialized 'guidepost' neurons that act as intermediate targets to shape the trajectory of pioneer axons [11]. In the CNS, similar experiments demonstrated that while certain pioneer growth cones rely on specialized glial cells for pathfinding choices, follower growth cones depend upon interactions with specific axons in the developing neuropil [see, e.g., ref. 12; reviewed in ref. 13]. This led to the 'labeled pathways' hypothesis which predicted axon fascicle-specific molecular labels required to direct the pathway choice of follower axons, an idea that echoed the chemoaffinity hypothesis made by Sperry in the 1960s [14]. Since cell ablation removes many gene products at once, the question of how complex these guidance labels might be remained open for the next wave of investigation. Although the relevance of this work in insects was met with some skepticism [see, e.g., ref. 15], time has validated the work and focussed the field on the underlying molecular mechanisms.

Genetics and the emergence of mechanism

The application of genetics to the problem of invertebrate axon guidance marked a transition away from grasshopper and other systems with large and easily manipulated neurons to *Drosophila* and the nematode *Caenorhabditis elegans*. The application of classical genetics to the study of neural development is generally credited to Poulson [16], for his work defining regions of the *Drosophila* genome necessary for neurogenesis. However, progress in the genetics of axon guidance is more recent. In *C. elegans*, the pioneering behavioral screens of Brenner [17] and subsequent genetic dissection [see, e.g., ref. 18] led to fundamental insights into the molecular mechanisms controlling the directional specificity of axons in vertebrates and invertebrates alike [reviewed in refs 19, 20]. Although Brenner's contemporary, Stent, argued that genetic approaches provide limited resources for the analysis of neural development [21], many axon guidance factors and receptors that have been defined by genetic analysis appear to play analogous roles in the vertebrate nervous system [see refs 22–24 for selected reviews]. The emerging picture demonstrates a high degree of molecular conservation between systems, thus validating the genetic approach. More recent work on the intracellular signaling mechanisms that interpret axon guidance cues, reviewed by Garrity in this issue, suggests that genetic tools will certainly help us understand the chain that links events at the cell surface with the dynamic cytoskeleton that shapes the movements of the growth cone. The power of genetic systems to test functional relationships between gene products in vivo is a significant asset for the study of complex signal transduction pathways.

Intermediate targets and the formation of axon pathways

As developing axons begin to extend into the cellular wilderness, the first guidance information they receive is not likely to come from the ultimate target cells which often lie in distant locations. Information that defines the direction of outgrowth, or that brings the axon from one turning point to the next, may be provided by various types of cells along the path. Many types of intermediate targets have been identified, including epithelial cell substrates, guidepost neurons, specialized glia, or other axons. A review by O'Connor in this issue explores the properties of such intermediate stepping stones in a variety of contexts. Then, reviews by Tear and Auld detail the roles of specialized non-neuronal cells in defining key guidance choices early in development. Finally, a review by Garcia-Alonso describes recent progress in the study of post-embryonic axon pathways, including the adult visual system and mechanosensory system. This survey of different axon guidance phenomena and some of the molecules that are known to function in the guidance machinery shows us that each different type of guidance choice presents a distinct set of molecular players. Some of these appear to regulate cell and substrate adhesion events, whereas others may activate or participate in signaling cascades that control the cytoskeletal motility machinery. Although certain gene functions overlap from one context to another, it seems clear that a diversity of mechanisms provides the growing axon with a rich source of guidance information. Many mechanisms also include a degree of functional redundancy, perhaps necessary to insure high fidelity in the final map of pathways and connections, or for aspects of guidance more subtle than we currently appreciate.

Final target recognition

The choice of a neuron for its appropriate synaptic partner marks the end of the axon journey through the embryo. Although many great minds have wrestled with the question of how each target presents a unique 'address' to attract the correct presynaptic mate, we are only now at the threshold of understanding this phenomenon in molecular terms. The most conspicuous of recent advances in this area have come from studies of topographic mapping within the vertebrate visual system [reviewed in ref. 25]. Here, graded signals control both the guidance of the primary retinal growth cone and the elaboration of axon collaterals to establish an accurate map of connections within the target region, satisfying the predictions of Sperry [14] made several decades ago. However, not all target cells are arrayed in a continuous field where each cell differs only incrementally from its neighbors. Some targets are arranged in discontinuous patterns, such as the primary targets in the vertebrate olfactory system, or the muscle targets of motor neurons in *Drosophila*. A review by Hoang and Chiba in this issue explores how the recognition process proceeds from initial recognition to a committed relationship between synaptic partners. Here again, *Drosophila* provides a promising system for detailed cellular analysis and gene discovery. The emerging picture predicts that target address labels will be composed of multiple components, with no single gene providing all of the necessary information to determine the appropriate choice. This view anticipates that each growth cone must be capable of integrating many simultaneous inputs in order to achieve the correct combination. How the interpretive machinery works is still a mystery, but one worthy of our attention as we move into the next century.

Prospects and conclusion

In the present era of molecular neurobiology, analysis of axon guidance mechanisms in vertebrate and invertebrate systems walk hand-in-hand towards a bright future of enlightenment. As we look into the future, continued application of genetic and cell biological approaches in invertebrate model organisms promises to yield rapid progress and synergy with work in vertebrate preparations. The increasing speed with which loss-of-function mutations can be obtained in the mouse provides an excellent opportunity for a true partnership between systems with powerful gene discovery technology, such as *Drosophila* and *C. elegans*, and a vertebrate nervous system that more closely parallels our own.

Acknowledgements. D.V.V. is supported by a McKnight Scholar Award, The Council for Tobacco Research, U.S.A. and NIH grant no. NS35909. L.J.L. is supported by the Whitehall Foundation.

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