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The Growth and Guidance of Axons

In the two preceding chapters, we saw how neurons are generated in appropriate numbers, at correct times, and in the right places. These early developmental steps set the stage for later events that direct neurons to form functional connections with target cells. To form connections, neurons have to extend long processes—axons and dendrites—which permit connectivity with postsynaptic cells and synaptic input from other neurons. In this chapter, we examine how neurons elaborate axons and dendrites and how axons are guided to their targets.

We begin the chapter by discussing how certain neuronal processes become axons and others dendrites. We then describe the growing axon, which may have to travel a long distance and ignore many inappropriate neuronal partners before terminating in just the right region and recognizing its correct synaptic targets. We consider the strategies by which the axon overcomes these challenges. Finally, we illustrate general features of axonal guidance by describing the development of two well-studied axonal pathways: one that conveys visual information from the retina to the brain and another that conveys cutaneous sensory information from the spinal cord to the brain.

Differences Between Axons and Dendrites Emerge Early in Development

The processes of neurons vary enormously in their length, thickness, branching pattern, and molecular architecture. Nonetheless, most neuronal processes fit into one of two functional categories: axons and dendrites. More than a century ago, Santiago Ramón y Cajal hypothesized that this distinction underlies the ability of neurons to transmit information in a particular direction, an idea he formalized as the law of dynamic polarization. Cajal wrote that "the transmission of the nerve impulse is always from the dendritic branches and the cell body to the axon." In the decades before electrophysiological methods were up to the task, this law provided a means of analyzing neural circuits histologically. Although exceptions have been found, Ramón y Cajal's law remains a basic principle

that relates structure to function in the nervous system and highlights the importance of knowing how neurons acquire their polarized form.

Progress in understanding how neuronal polarization occurs comes in large part from studies of neurons taken from the rodent brain and grown in tissue culture. Hippocampal neurons grown in isolation develop processes reminiscent of those seen in vivo: a single, long, cylindrical axon and several shorter, tapered dendrites (Figure 47–1A). As cytoskeletal and synaptic proteins are differentially targeted to these components, axons and dendrites acquire distinctive molecular profiles. For example, a particular form of the Tau protein is localized in axons and the MAP2 protein in dendrites (Figure 47–1B)

Cultured neurons are especially useful for developmental studies because they initially show no obvious sign of polarization and acquire their specialized features gradually in a stereotyped sequence of cellular steps. This sequence begins with extension of several short processes, each equivalent to the others. Soon thereafter, one process is established as an axon and the remaining processes acquire dendritic features (Figure 47–1A).

How does this occur? Cytoskeletal proteins that maintain elongated processes and drive growth are central to this process. If the actin filaments in an early neurite are destabilized, the cytoskeleton becomes reconfigured in a way that commits the neurite to becoming the axon; secondarily, the remaining neurites react by becoming dendrites. If the nascent axon is removed, one of the remaining neurites quickly assumes an axonal character. This sequence suggests that axonal specification is a key event in neuronal polarization and that signals from newly formed axons both suppress the generation of additional axons and promote dendrite formation.

The nature of the axonally derived signal that represses other axons is not known, but some insight into signals that control cytoskeletal arrangements has come from the study of a group of proteins encoded by the *Par* complex genes. As first shown in the nematode worm *Caenorhabditis elegans*, Par proteins are involved in diverse aspects of cytoskeletal reorganization, including the polarization of neuronal processes. Mammalian forebrain neurons lacking *Par3*, *Par4*, *Par6*, or relatives of *Par1* grow multiple processes that are intermediate in length between axons and dendrites and bear markers of both processes (Figure 47–1B).

Although neurons grown in culture are similar to those in the brain, they are deprived of key extrinsic cues and signals. Cultured neurons become randomly arranged with respect to each other, whereas in many regions of the developing brain, neurons line up in rows, with their dendrites pointing in the same direction (Figure 47–2A). As the neurons migrate to their destinations (Chapter 46), axons and dendrites often grow as extensions of their trailing and leading processes, respectively. This difference in vivo and in vitro implies that extrinsic signals regulate the polarization machinery. In the developing brain, the local release of semaphorins and other axonal guidance factors, discussed later in the chapter, may help to orient dendrites (Figure 47–2C). The job of the Par protein complex is to link these extracellular signals to the cellular machinery that rearranges the cytoskeleton, a process achieved in part through the regulation of proteins that modify actin or tubulin function. In fact, both the Tau protein in axons and the MAP2 protein in dendrites associate with and affect microtubules. Cytoskeletal differences also contribute to other mechanisms that amplify distinctions between axons and dendrites, such as polarized trafficking of molecules and generation of a specialized initial segment in axons.

If local signals are needed to polarize neurons in the brain, how is polarity established in the uniform environment of a tissue culture? One possible explanation is that minor variations in the intensity of signaling within a neuron, or in signals from its immediate environment, will activate Par proteins in one small domain of the neuron, triggering the nearest cell process to become an axon. If, by happenstance, one process grows slightly faster than its neighbors or encounters an environment that speeds neurite extension (Figure 47–2B), its chances of becoming an axon increase markedly. Presumably, this proto-axonal process emits signals that decrease the chance of other processes following suit, forcing them to become dendrites.

Dendrites Are Patterned by Intrinsic and Extrinsic Factors

Once polarization occurs, dendrites grow and mature, acquiring the structural features that distinguish them from axons. Nascent dendrites form branched arbors, with their branches generally being more numerous and closer to the cell body than those of axons. In addition, small protrusions called spines extend from the distal branches of many dendrites. Finally, some dendritic branches are retracted or "pruned" to give the arbor its final and definitive shape (Figure 47–3).

Although the core features of dendrite formation are common to many neurons, there is striking variation in their number, shape, and branching pattern among neuronal types. Indeed, the shape of dendritic arbors is one of the main ways in which neurons can

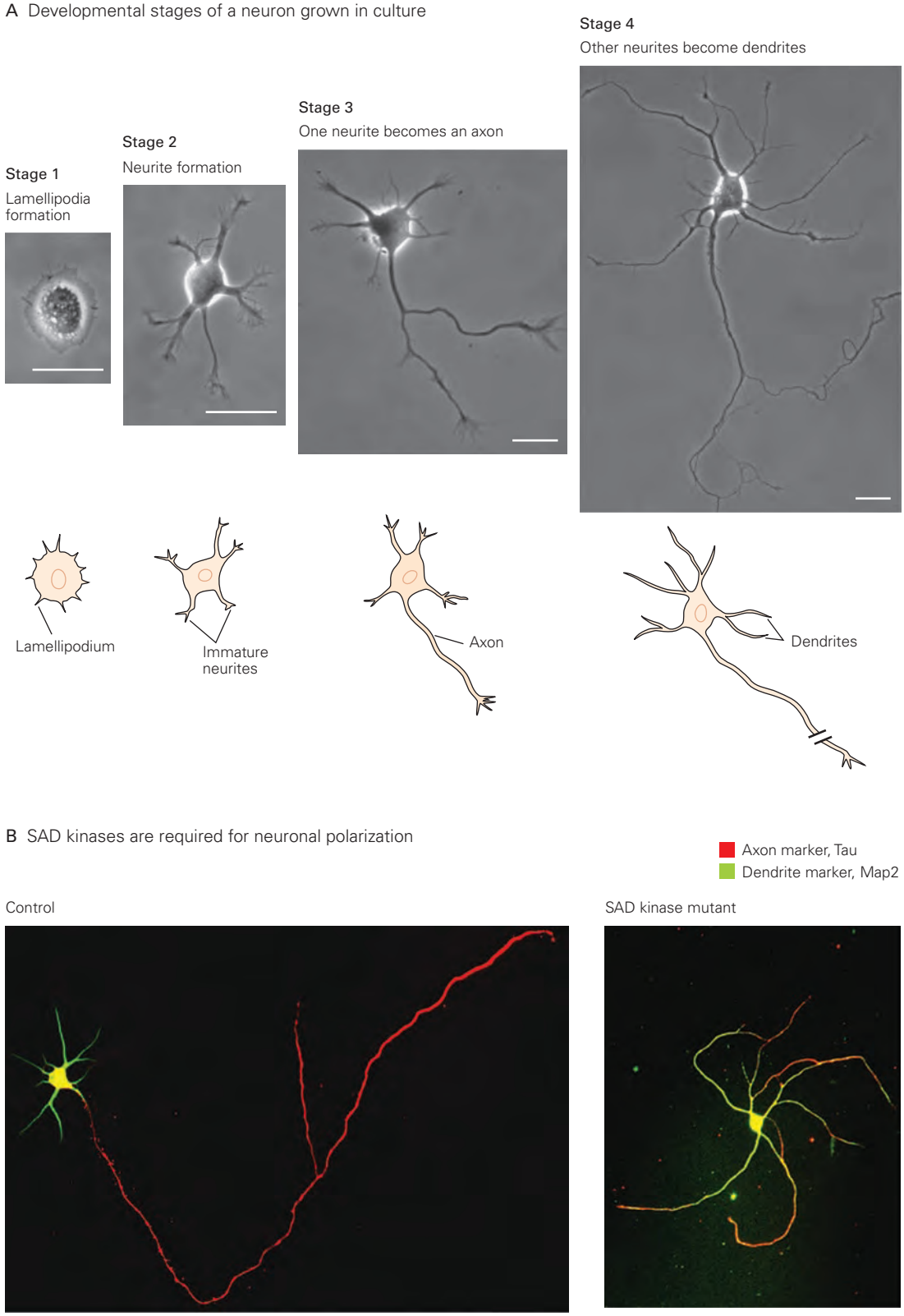

Figure 47–1 The differentiation of axons and dendrites marks the emergence of neuronal polarity.

A. Four stages in the polarization of a hippocampal neuron grown in tissue culture. (Adapted, with permission, from Kaech and Banker 2006. Copyright © 2007 Springer Nature.)

B. Hippocampal neurons grown in culture possess multiple short, thick dendrites that are enriched in the microtubule-associated protein MAP2. They also possess a single long axon that is marked by a dephosphorylated form of the microtubule-associated protein tau (*left*). A cultured neuron isolated from a mutant mouse lacks expression of a *Par* family gene (SAD kinase). The neuron generates neurites that express both tau and MAP2, markers of axons and dendrites, respectively. The length and diameter of these neurites are intermediate in size between those of axons and dendrites (*right*). (Reproduced, with permission, from Kishi et al. 2005.)

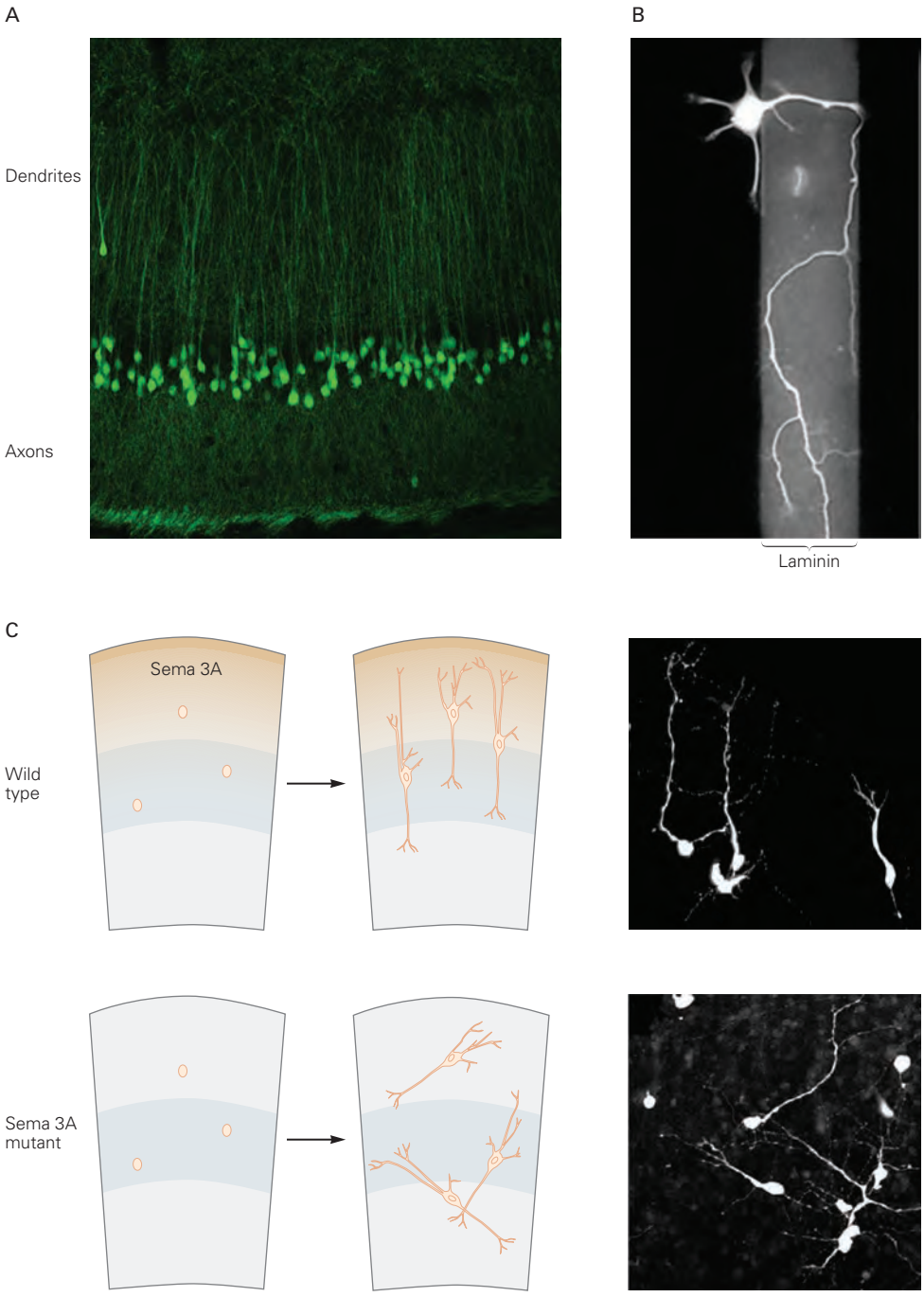

Figure 47–2 Extracellular factors determine whether neuronal processes become axons or dendrites.

A. Cortical pyramidal neurons in vivo display a common axonal and dendritic orientation.

B. Neurons growing on laminin acquire polarity. When a cortical neuron extends a process from a less attractive substrate onto laminin, the process grows faster and usually becomes an axon. (Image reproduced, with permission, from Paul Letourneau.)

C. In the developing neocortex, semaphorin-3A (**Sema 3A**) is secreted by cells near the pial surface. Semaphorin-3A is an attractant for growing dendrites, helping to establish neuronal polarity and orientation. The parallel orientation of cortical pyramidal neurons is disrupted in mutant mice lacking functional semaphorin-3A. (Reproduced, with permission, from Polleux, Morrow, and Ghosh 2000. Copyright © 2000 Springer Nature.)

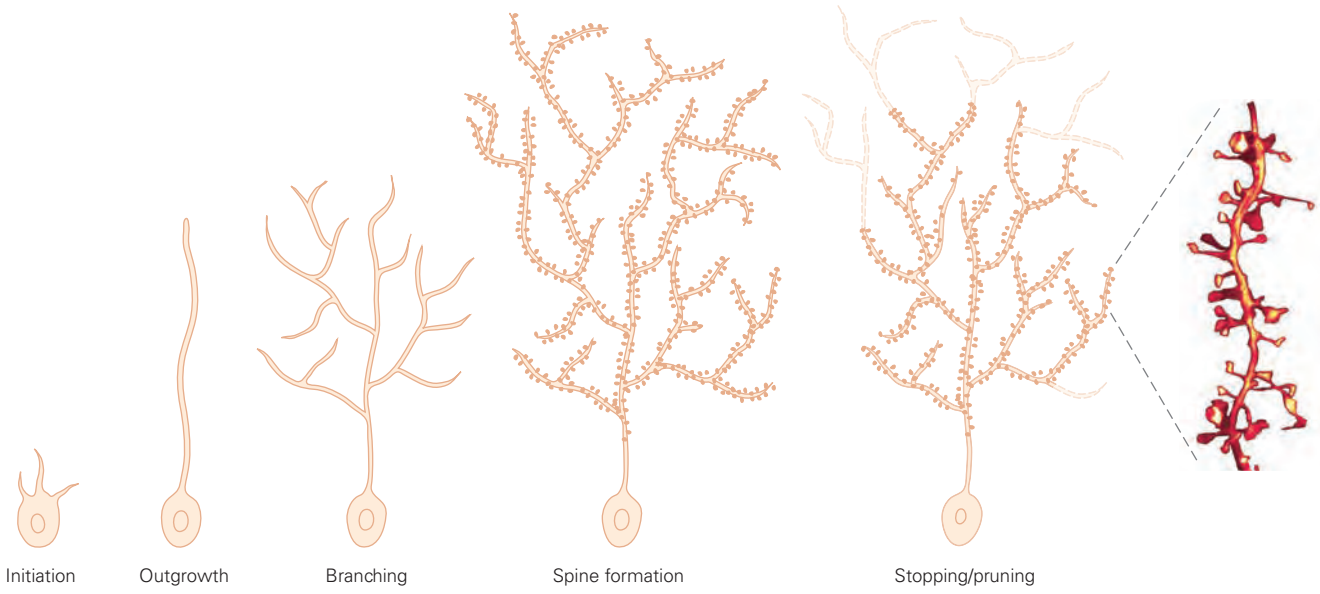

Figure 47–3 Dendritic branching develops in a series of steps. The outgrowth of dendrites involves the formation of elaborate branches from which spines develop. Certain branches and spines are later pruned to achieve the mature pattern of dendrite arborization. (Image of spines at right reproduced, with permission, from Stefan W. Hell.)

be classified. Cerebellar Purkinje cells can be distinguished from granule cells, spinal motor neurons, and hippocampal pyramidal neurons simply by looking at the pattern of their dendrites. These variations are critical for the distinct functions of different neuronal types. For example, the size of a dendritic arbor and the density of its branches are main determinants of the number of synapses it receives.

How is dendritic pattern established? Neurons must have intrinsic information about their shape because the patterns in tissue culture are strikingly reminiscent of those in vivo (Figure 47–4). The transcriptional programs that specify neuronal subtype (Chapter 46) presumably also encode information about neuronal shape. In both invertebrates and vertebrates, some transcription factors are selectively expressed by specific neuronal types and appear to be devoted to controlling the size, shape, and complexity of their dendritic arbors. They do so by coordinating the expression of downstream genes, including those encoding components of the cytoskeletal apparatus and membrane proteins that mediate interactions with neighboring cells.

A second mechanism for establishing the pattern of dendritic arbors is the recognition of one dendrite by others of the same cell. In some neurons, dendrites are spaced evenly with respect to each other, an arrangement that allows them to sample inputs efficiently without major gaps or clumps (Figure 47–5A). In many cases, this process, called self-avoidance, occurs through a mechanism in which branches belonging to the same neuron repel each other. Several cell-surface adhesion molecules have now been found that mediate self-avoidance by interacting in a way that results in repulsion (Figure 47–5D). Although it seems counterintuitive that an adhesive interaction between adjacent membranes would lead to repulsion rather than attachment, the consequences of most intercellular interactions are determined by the signaling they initiate rather than by adhesion per se, as we will see later in this chapter.

The dendrites of neighboring neurons also provide cues. In many cases, the dendrites of a particular neuron type cover a surface with minimal overlap, a spacing pattern called *tiling* (Figure 47–5B). The tiling of dendrites is conceptually related to self-avoidance, but in tiling, the inhibitory dendritic interactions are among neurons of a particular type, whereas in self-avoidance, they are among sibling dendrites of a single neuron. Tiling allows each class of neuron to receive information from the entire surface or area it innervates. Tiling of a region by the dendrites of one class of neuron also avoids the confusion that could arise if the dendrites of many different neurons occupied the same area.

A particularly interesting situation is one in which dendrites engage in self-avoidance but synapse on the dendrites of other cells of the same type. In this situation, dendrites face the challenging task of distinguishing nominally identical dendrites from dendrites of

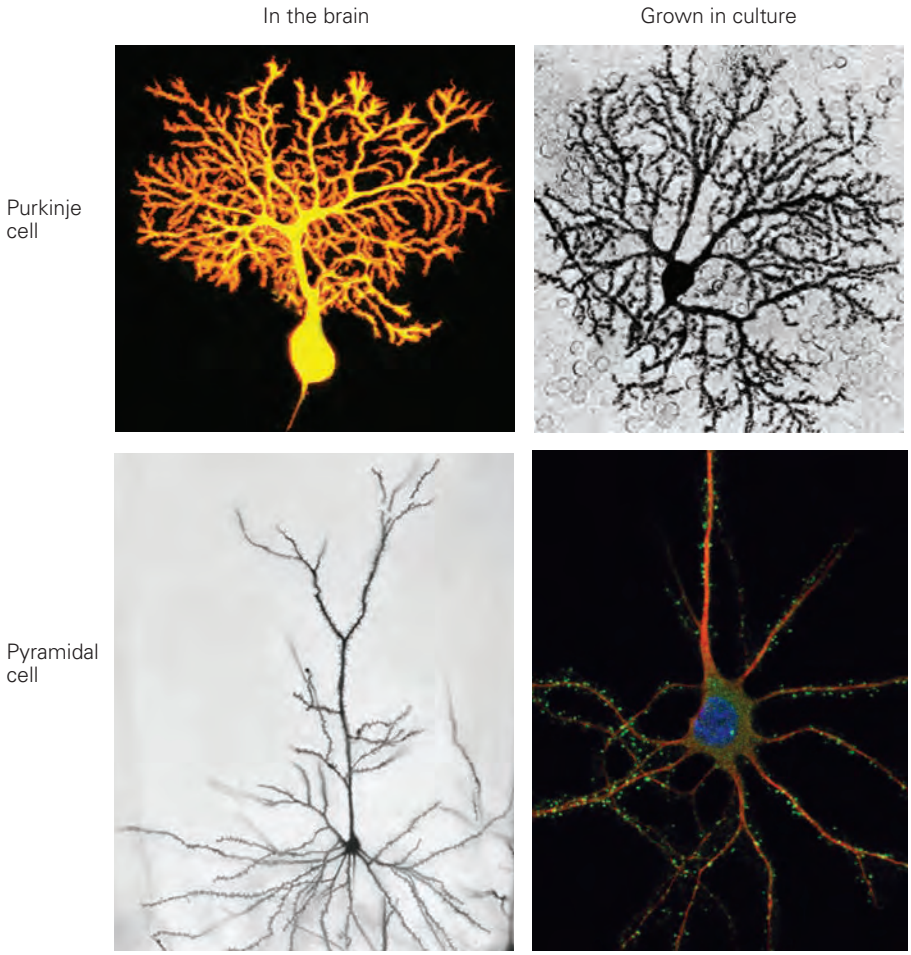

Figure 47–4 The morphologies of neurons are preserved in dissociated cell culture. Cerebellar Purkinje neurons and hippocampal pyramidal neurons have distinctive patterns of dendritic branching. These basic patterns are recapitulated when these two classes of neurons are isolated and grown in dissociated cell culture. (Image upper left: Dr. David Becker; upper right reproduced, with permission, from Yoshio Hirabayashi; lower left reproduced, with permission, from Terry E. Robinson; lower right reproduced, with permission, from Kelsey Martin.)

nominally identical cells (Figure 47–5C). Two groups of molecules have been identified that mediate this self-/non–self-discrimination: clustered protocadherins in mammals and DS-CAMs in *Drosophila*. Although they are unrelated structurally, they share several features (Figure 47–5D).

First, both are encoded by large, complex genes that generate large numbers of isoforms. *Drosophila* Dscam1 encodes around 38,000 distinct proteins through alternative splicing, and the clustered protocadherins encode around 60 proteins that can assemble into thousands of distinct multimers. Second, nearly all of the isoforms bind homophilically; for example, protocadherin γa1 on the surface of one dendrite binds well to protocadherin γa1 on a neighboring membrane, but poorly if at all to other isoforms. Third, in ways that remain incompletely understood, each neuron within a population expresses a random subset of all possible Dscam1 or protocadherin isoforms. Given the large number of isoforms, it is unlikely that individual neurons express identical sets of isoforms on their cell surface. The upshot is that dendrites of each neuron in a population bind homophilically to sibling dendrites, leading to repulsion and self-avoidance, whereas they bind poorly to dendrites of neighboring neurons, enabling other recognition systems to foster synaptogenesis.

Together, the mechanisms we have described, and many others, establish an overall arborization pattern through a combination of intrinsic and extracellular mechanisms. For dendrites, the extrinsic patterning signals determine neuronal morphology. For axons, which we consider next, the signals guide the axons to their targets.

The Growth Cone Is a Sensory Transducer and a Motor Structure

Once an axon forms, it begins to grow toward its synaptic target. The key neuronal element responsible for axonal growth is a specialized structure at the tip of the axon called the *growth cone.* Both axons and dendrites

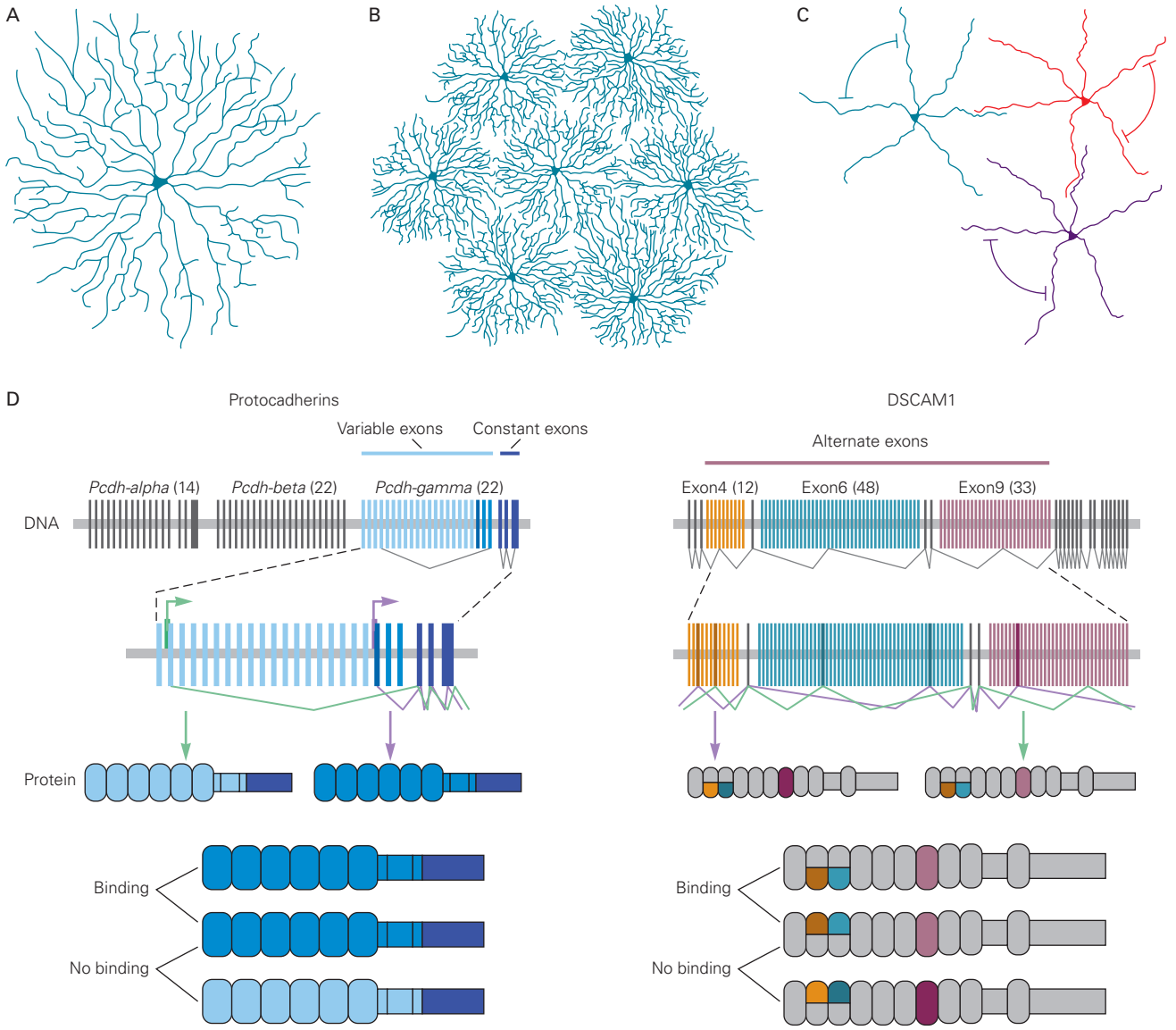

Figure 47–5 Interactions among dendritic branches pattern dendritic arbors.

A. Self-avoidance among sibling dendrites leads to even spacing of branches, minimizing gaps and clumps. In retinal starburst amacrine cells, self-avoidance fails when gamma protocadherins are lost.

B. Tiling of dendrites is conceptually similar to self-avoidance but applies to groups of neurons. It ensures that neighboring neurons of a single type cover territory efficiently.

C. Self-/non-self–discrimination allows sibling dendrites to avoid each other while interacting freely with dendrites of other neurons of the same type.

D. Generation of numerous adhesion molecules from a single genomic complex by promoter choice at the mouse clustered protocadherin (**Pcdh**) locus (*left*) and by alternative splicing at the *Drosophila* DSCAM1 locus (*right*).

use growth cones for elongation, but those linked to axons have been studied more intensively.

Ramón y Cajal discovered the growth cone and had the key insight that it was responsible for axonal pathfinding. With static images alone for inspiration (Figure 47–6A), he envisioned the growth cone to be "endowed with exquisite chemical sensitivity, rapid ameboid movements and a certain motive force, thanks to which it is able to proceed forward and overcome obstacles met in its way . . . until it reaches its destination."

Many studies over the past century have confirmed Ramón y Cajal's intuition. We now know

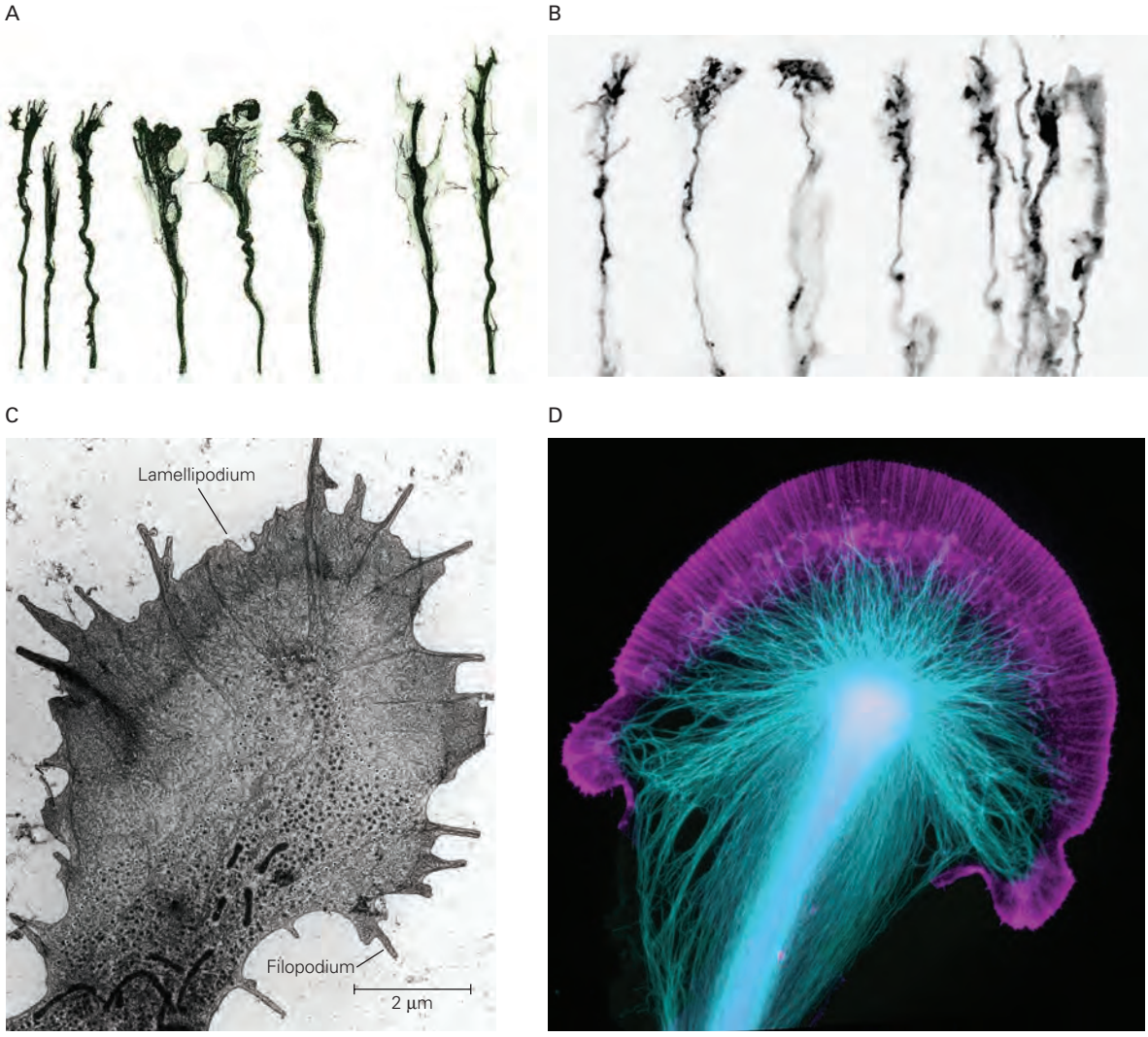

Figure 47–6 Neuronal growth cones.

A. Drawings of growth cones by Santiago Ramón y Cajal, who discovered these cellular structures and inferred their function.

B. Growth cones visualized in dye-labeled retinal ganglion neurons in the mouse. Note the similarities with Cajal's drawings. (Reproduced, with permission, from Carol Mason and Pierre Godement.)

C. The three main domains of the growth cone—filopodia, lamellipodia, and a central core—are shown by whole-mount scanning electron microscopy. (Reproduced, with permission, from Bridgman and Dailey 1989. Permission conveyed through Copyright Clearance Center, Inc.)

D. The growth cone of a neuron from *Aplysia* in which actin and tubulin have been visualized. Actin (**purple**) is concentrated in lamellipodia and filopodia, whereas tubulin and microtubules (**aquamarine**) are concentrated in the central core. (Reproduced, with permission, from Paul Forscher and Dylan Burnette.)

that the growth cone is both a sensory structure that receives directional cues from the environment and a motor structure whose activity drives axon elongation. Ramón y Cajal also pondered "what mysterious forces precede the appearance of these processes . . . promote their growth and ramification . . . and finally establish those protoplasmic kisses . . . which seem to constitute the final ecstasy of an epic love story." In more modern and prosaic terms, we now know that the growth cone guides the axon by transducing positive and negative cues into signals that regulate the cytoskeleton, thereby determining the course and rate of axonal growth toward its targets, where it will form synapses.

Growth cones have three main compartments. Their *central core* is rich in microtubules, mitochondria, and other organelles. Long slender extensions called

filopodia project from the body of the growth cone. Between the filopodia lie *lamellipodia*, which are also motile and give the growth cone its characteristic ruffled appearance (Figure 47–6C,D).

Growth cones sense environmental signals through their filopodia: rod-like, actin-rich, membrane-limited structures that are highly motile. Their surface membranes bear receptors for the molecules that serve as directional cues for the axon. Their length—tens of micrometers in some cases—permits the filopodia to sample environments far in advance of the central core of the growth core. Their rapid movements permit them to compile a detailed inventory of the environment, and their flexibility permits them to navigate around cells and other obstacles.

When filopodia encounter signals in the environment, the growth cone is stimulated to advance, retract, or turn. Several motors power these orienting behaviors. One source of power is the movement of actin along myosin, an interaction similar to the one that powers the contraction of skeletal muscle fibers, although the actin and myosin of neurons are different from those in muscle. The assembly of actin monomers into polymeric filaments also contributes a propulsive force for filopodial extension. As the actin filaments are constantly depolymerized at the base of filopodia, the balance of polymerization and depolymerization enables the filopodia to move forward without becoming longer. Depolymerization slows during periods of growth cone advance, leading to greater net forward motion. The movement of membranes along the substrate provides yet another source of forward motion.

The contribution of each type of molecular motor to the advance of the growth cone is likely to vary from one situation to another. Nevertheless, the final step involves the flow of microtubules from the central core of the growth cone into the newly extended tip, thus moving the growth cone ahead and leaving in its wake a new segment of axon. New lamellipodia and filopodia form in the advancing growth cone and the cycle repeats (Figure 47–7).

Accurate pathfinding can occur only if the growth cone's motor action is linked to its sensory function. Therefore, it is crucial that the recognition proteins on the filopodia are signal-inducing receptors and not merely binding moieties that mediate adhesion. The binding of a ligand to its receptor affects growth in diverse ways. In some cases, it engages the cytoskeleton directly, through the intracellular domain of receptors (Figure 47–7). Integrin receptors couple to actin in growth cones when they bind molecules associated with the surface of adjoining cells or the extracellular matrix, thereby influencing motility.

Of equal if not greater importance is the ability of ligand binding to stimulate the formation, accumulation, and even breakdown of soluble intracellular molecules that function as second messengers. These second messengers affect the organization of the cytoskeleton, and in this way regulate the direction and rate of movement of the growth cone.

One important second messenger is calcium. The calcium concentration in growth cones is regulated by the activation of receptors on filopodia, and this affects the organization of the cytoskeleton, which in turn modulates motility. Growth cone motility is optimal within a narrow range of calcium concentrations, called a *set point*. Activation of filopodia on one side of the growth cone leads to a concentration gradient of calcium across the growth cone, providing a possible basis for changes in the direction of growth.

Other second messengers that link receptors and motor molecules include cyclic nucleotides, which modulate the activity of enzymes such as protein kinases, protein phosphatases, and rho-family guanosine triphosphatases (GTPases). In turn, these messengers and enzymes regulate the activity of proteins that regulate the polymerization and depolymerization of actin filaments, thereby promoting or inhibiting axonal extension.

The critical role of intracellular signals in growth cone motility and orientation can be demonstrated using embryonic neurons grown in culture. Application of growth factors to one side of a growth cone activates receptors locally and leads to extension and turning of the growth cone toward the source of the signal. In essence, the factor attracts the growth cone. Yet when cyclic adenosine monophosphate (cAMP) levels in the neuron are decreased, the same stimulus acts as a repellent and the growth cone turns away from the signal (Figure 47–8A). Other repulsive factors can become attractive when levels of the second messenger cyclic guanosine 3′,5′-monophosphate (cGMP) are raised.

Recently, another mechanism for coupling guidance molecules to growth cone behavior has come to light. It was long thought that all neuronal protein synthesis occurs in the cell body, but we now know that growth cones (as well as some dendrites) contain the machinery for protein synthesis, including a subset of messenger RNAs. Initial evidence that these molecules play an important role came from experiments in which axons were severed from their parent cell body. The growth cones continued to advance for a few hours; they could be stimulated to turn toward or away from local depots of guidance molecules, and these behaviors were abolished by inhibitors of protein