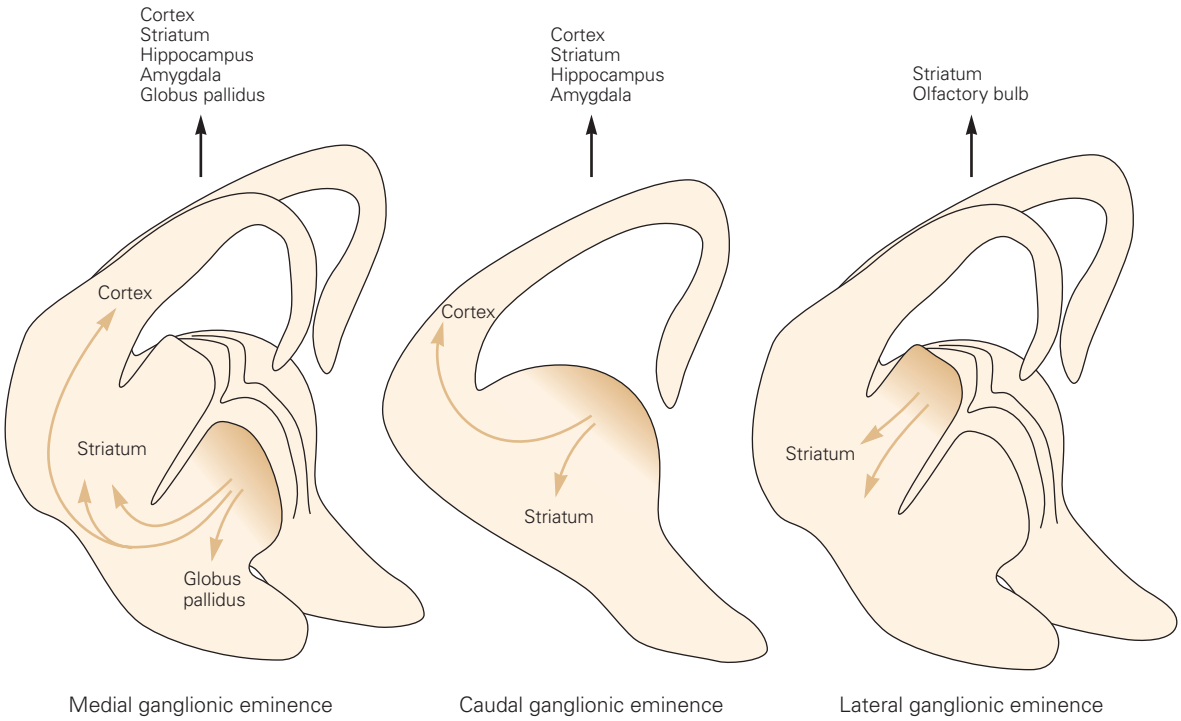

Figure 46–8 Forebrain interneurons are generated in the ventral telencephalon and migrate tangentially to the cerebral cortex. Neurons generated in the ganglionic eminences migrate to and settle in many regions of the forebrain, where they differentiate into interneurons. Cortical interneurons arise from medial and caudal ganglionic eminences. Other cells generated in these regions migrate in other directions, populating the hippocampus, striatum, globus pallidus, and amygdala with interneurons. The lateral ganglionic eminence generates cells that migrate to the striatum and the olfactory bulb. Cells migrating to the bulb use neighboring migrating cells as substrates for migration, a process called chain migration. (Adapted, with permission, from Bandler, Mayer, and Fishell 2017. Copyright © 2017 Elsevier Ltd.)

Nonetheless, it is now clear that cortical neurons originate from two sources: excitatory neurons from the cortical ventricular zone and interneurons from the ganglionic eminences.

Interneurons in other forebrain structures also arise from the ganglionic eminences, as well as a few other subcortical sites such as the preoptic area. Cells migrating caudally from the medial and caudal eminences populate the hippocampus, while cells migrating ventrolaterally from these regions populate the basal ganglia. In contrast, neurons generated in the lateral ganglionic eminence migrate rostrally and contribute the periglomerular and granule interneurons of the olfactory bulb. In this rostral migratory stream, neurons use neighboring neurons as substrates for migration (chain migration). In the adult brain, neurons that follow the rostral migratory stream originate instead in the subventricular zone of the striatum.

Transcription factors control the character of ganglionic eminence neurons. The homeodomain proteins Dlx1 and Dlx2 are expressed by cells in the ganglionic eminences. In mice lacking Dlx1 and Dlx2 activity, the resultant perturbation of neuronal migration leads to a profound reduction in the number of GABAergic interneurons in the cortex. Other transcription factors are responsible for differences among ganglionic eminences. For example, Nkx2.1 is selectively expressed by cells in the medial ganglionic eminence. In its absence, interneurons generated in this region take on characteristics of those normally generated in the lateral and caudal ganglionic eminences. Yet other transcription factors specify the distinct characteristics of subpopulations of neurons within each ganglionic eminence.

One of the main features that these transcription factors specify is the migratory path that the newborn interneurons take. A host of soluble and cell surface factors produced by cells in and near the ganglionic eminences provide repulsive cues that lead to expulsion of cells from the ventricular zone, so-called motogenic (movement-promoting) cues that speed their migration and attractive cues that direct them to their targets. These factors include slits, semaphorins, and ephrins, all of which we will encounter in Chapter 47 as molecules that guide axons to their targets.

Neural Crest Cell Migration in the Peripheral Nervous System Does Not Rely on Scaffolding

The peripheral nervous system derives from neural crest stem cells, a small group of neuroepithelial cells at the boundary of the neural tube and epidermal ectoderm. Soon after their induction, neural crest cells are transformed from epithelial to mesenchymal cells and begin to detach from the neural tube. They then migrate to many sites throughout the body (Figure 46–9). Neural crest cell migration does not rely on scaffolding (ie, radial glial cells or preexisting axon tracts) and thus is called free migration. This form of neuronal migration requires significant cytoarchitectural and cell adhesive changes and differs from most of the migratory events in the central nervous system.

Neural crest migration is promoted and guided by several families of secreted factors. For example, bone morphogenetic proteins (BMPs), which are critical for neural crest induction at an earlier stage (Chapter 45), are required for neural crest migration at later stages. Exposure of neural epithelial cells to BMPs triggers molecular changes that convert epithelial cells to a mesenchymal state, causing them to delaminate from the neural tube and migrate into the periphery. BMPs trigger changes in neural crest cells by inducing expression of transcription factors, notably the zinc finger proteins snail, slug, and twist, which have a conserved role in promoting epithelial-to-mesenchymal transitions. These transcription factors direct expression of proteins that regulate the properties of the cytoskeleton as well as enzymes that degrade extracellular matrix proteins. These enzymes give neural crest cells the ability to break down the basement membrane surrounding the epithelium of the neural tube, permitting them to embark on their migratory journey into the periphery.

As neural crest cells begin to delaminate, their expression of cell adhesion molecules changes. Alterations in expression of adhesive proteins, notably cadherins, permit neural crest cells to loosen their adhesive contacts with neural tube cells and begin the delamination process. Neural crest cells also begin to express integrins, receptors for extracellular matrix proteins such as laminins and collagens that are found along peripheral migratory paths.

The first structures encountered by migrating neural crest cells are somites, epithelial cells that later give rise to muscle and cartilage. Neural crest cells pass through the anterior half of each somite but avoid the posterior half (Figure 46–9A). The rostral channeling of migratory neural crest cells is imposed by ephrin B proteins, which are concentrated in the posterior half of each somite. Ephrins provide a repellant signal that interacts with EphB class tyrosine kinase receptors on neural crest cells to prevent their invasion. Neural crest cells that remain within the anterior sclerotome of the somite differentiate into sensory neurons of the dorsal root ganglia; those that migrate around the dorsal region of the somite approach the skin and give rise to melanocytes.

Differentiation of the neural crest into its various derivatives depends on complex interactions between the distinct cues that cells receive along their journey and intrinsic predispositions that vary along the rostrocaudal axis. Development of sensory neurons is initiated at the time the cells emigrate from the neural tube. The cells are exposed to signals from the dorsal neural tube and somites that induce expression of neurogenin, a transcription factor of the bHLH family, which in turn promotes a sensory fate. Subsequent influences diversify the neurons into multiple sensory types, such as nociceptive and proprioceptive neurons (Figure 46–10). In contrast, those neural crest cells that follow a more medial and ventral migratory path are exposed to BMPs secreted from the dorsal aorta. They express the bHLH factor Mash1, which leads to their differentiation into sympathetic neurons.

Structural and Molecular Innovations Underlie the Expansion of the Human Cerebral Cortex

No mice or monkeys are reading this book. This is in large part because the human brain is different from that of even our closest relatives, both qualitatively and quantitatively. Yet most studies of mammalian neural development have been carried out on mice, whose brain contains approximately 1,000-fold fewer neurons than those of the human brain and 100-fold fewer than the best-studied nonhuman primate, the rhesus macaque. Recently, however, new methods are making it possible to elucidate some of the molecular and structural features that lead to the expansion of the human brain and particularly the human cerebral cortex.

Classical anatomical studies made clear that the primate cortex has not only a far larger size and thickness than that of rodents but also more discrete areas and more layers (Figure 46–11A). In addition, the packing density of neurons is higher in primates than in mice, so the difference in neuronal number is greater than would be expected from size alone. One main contributor to the expansion in primates is a large pool of neuronal progenitors. Many of these progenitors are a second type of radial glial cell, called the outer radial

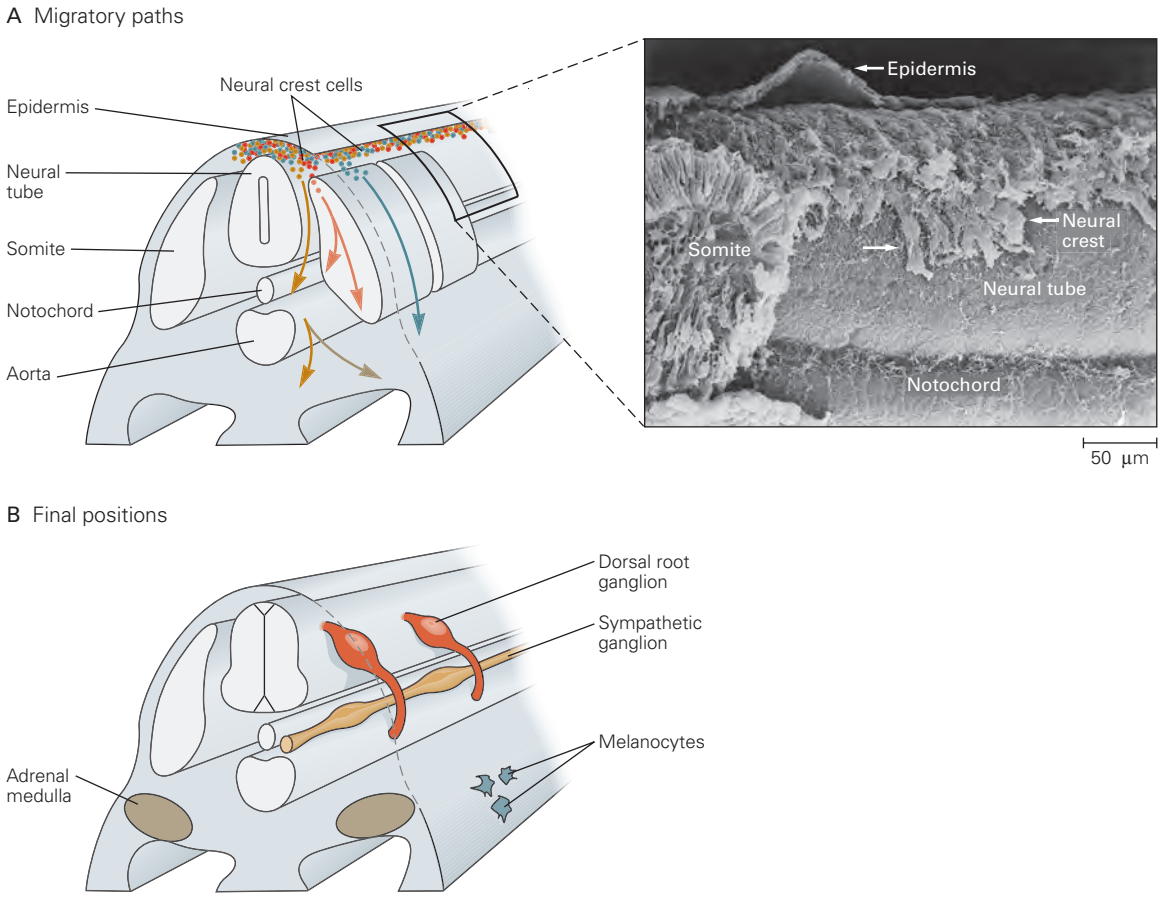

Figure 46–9 Neural crest cell migration in the peripheral nervous system.

A. A cross section through the middle part of the trunk of a chick embryo shows the main pathways of neural crest cells. Some cells migrate along a superficial pathway, just beneath the ectoderm, and differentiate into pigment cells of the skin. Others migrate along a deeper pathway that takes them through the somites, where they coalesce to form dorsal root sensory ganglia. Still others migrate between the neural tube and somites, past the dorsal aorta. These cells differentiate into sympathetic ganglia and adrenal medulla. The scanning electron micrograph shows neural crest cells migrating away from the dorsal surface of the neural tube of a chick embryo. (Micrograph reproduced, with permission, from K. Tosney.)

B. Neural crest cells reach their final settling positions where they complete differentiation.

glial cell to distinguish it from the canonical or inner radial glia described above. Outer radial glia, unlike inner radial glia, lack contact with the ventricular surface and exhibit molecular differences from inner radial glia. However, they are capable of generating neurons and serving as a migratory guide. The massive increase in their number in primates, and particularly humans, provides a partial explanation for the increase in the number of neurons in the human cerebral cortex.

How can human-specific developmental features be analyzed experimentally? New methods of molecular analysis are making it possible to compare the proteins, transcripts, and genes of humans with those of our close relatives, resulting in the discovery of intriguing specializations. However, hypotheses derived from these findings are difficult to test: Most of the developmental studies we describe in these chapters cannot be performed on humans, and even nonhuman primates are difficult subjects for developmental analysis. A possible solution is the recently devised "organoid" culture system.

Cells from adult skin can be reprogrammed to become multipotential progenitors called induced pluripotent stem cells (iPSCs) by methods that we will discuss in Chapter 50. When placed into culture under carefully controlled conditions and allowed to expand in three dimensions (quite unlike conventional two-dimensional cultures), they proliferate and self-organize into structures that resemble the developing forebrain and exhibit species-specific features

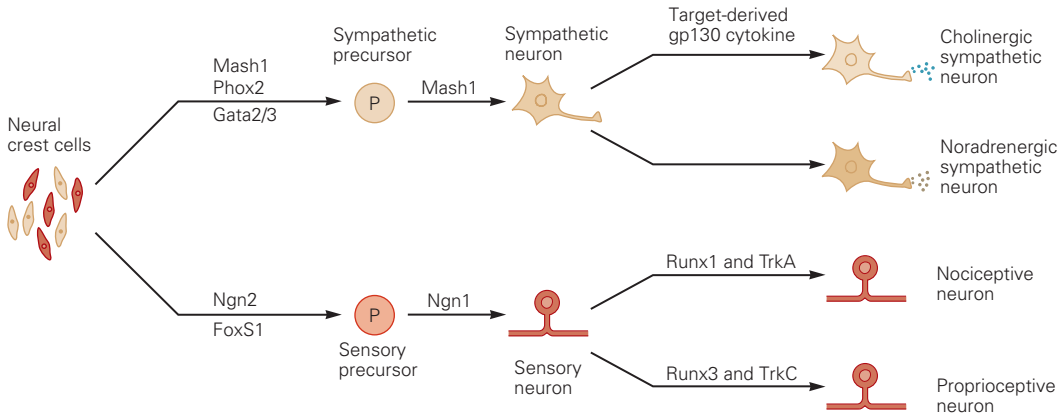

Figure 46–10 **Neural crest cells differentiate into sympathetic and sensory neurons.** The neuronal fates of trunk neural crest cells are controlled by transcription factor expression. Expression of the basic helix-loop-helix (bHLH) protein Mash1 directs neural crest cells along a sympathetic neuronal pathway. Sympathetic neurons can acquire noradrenergic or cholinergic transmitter phenotypes depending on the target cells they innervate and the level of gp130 cytokine signaling (see Figure 46–13). Two bHLH proteins, neurogenin-1 and -2, direct neural crest cells along a sensory neuronal pathway. Sensory neurons that express the transcription factor Runx1 and the tyrosine kinase receptor TrkA become nociceptors; those that express Runx3 and TrkC become proprioceptors. (Abbreviations: **Ngn-1**, neurogenin-1; **Ngn-2**, neurogenin-2.)

(Figure 46–11B). Most notably, organoids from human cells contain a bilayered, large subventricular zone with numerous outer radial glia, whereas organoids from mouse cells contain a smaller subventricular zone containing predominately conventional or inner radial glia. These organoids can be used to elucidate the development of at least some early aspects of human cortical development.

Additional applications abound. One is to obtain iPSCs from patients with brain disorders. Organoids derived from such patients have features that may lead to cortical malformations such as lissencephaly (see Figure 46–5). The hope is that these organoids can be used to elucidate disease mechanisms and eventually test therapies. A second application is to compare organoids derived from chimpanzee and human iPSCs. This comparison provides a novel means of investigating the most recent evolutionary innovations that separate us from our closest living relatives.

Intrinsic Programs and Extrinsic Factors Determine the Neurotransmitter Phenotypes of Neurons

Neurons continue to develop after they have migrated to their final position, and no aspect of their later differentiation is more important than the choice of chemical neurotransmitter. Neurons that populate the brain use two major neurotransmitters: The amino acid L-glutamate is the major excitatory transmitter, whereas γ-aminobutyric acid (GABA) is the major inhibitory transmitter. Some spinal cord neurons use another amino acid, glycine, as their inhibitory transmitter. In the peripheral nervous system, sensory neurons use glutamate, motor neurons use acetylcholine, and autonomic neurons use acetylcholine or norepinephrine. Smaller numbers of neurons use other transmitters, such as serotonin and dopamine. The choice of neurotransmitter determines which postsynaptic cells a neuron can talk to and what it can say.

Neurotransmitter Choice Is a Core Component of Transcriptional Programs of Neuronal Differentiation

Distinct molecular programs are used to establish neurotransmitter phenotype in different brain regions and neuronal classes. We shall illustrate the general strategy for assignment of amino acid neurotransmitter phenotypes by focusing on neurons in the cerebral cortex and cerebellum.

The cerebral cortex contains glutamatergic pyramidal neurons that are generated within the cortical plate and rely on the bHLH factors neurogenin-1 and neurogenin-2 for their differentiation. In contrast, as discussed earlier in the chapter (see Figure 46–8), most GABAergic inhibitory interneurons migrate into the cortex from the ganglionic eminences; their inhibitory transmitter character is specified by the bHLH protein

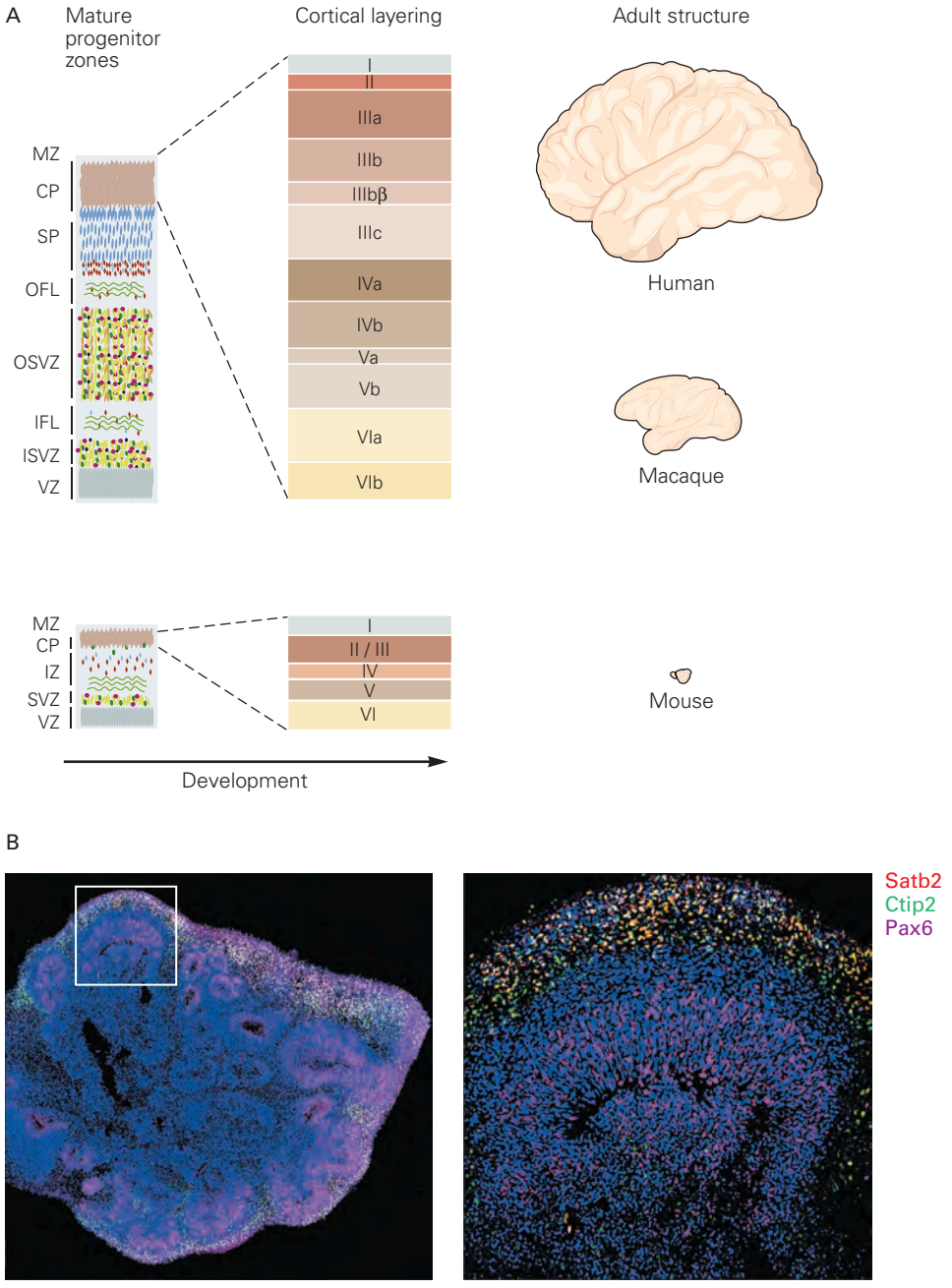

Figure 46–11 Expansion of the proliferative zones contributes to cortical specialization in humans and other primates.

A. The size of the neuroepithelium is initially small in both rodents and humans, but their relative size differs dramatically as development proceeds, owing to increased self-renewal rates and larger numbers of progenitors in humans. The primate subventricular zone is greatly enlarged compared to the mouse and becomes subdivided into inner and outer regions, which contain large populations of radial glial cells, both of which generate neurons. In mice, nearly all radial glial cells are of the inner type. (Abbreviations: **CP**, cortical plate; **IFL**, inner fiber layer; **ISVZ**, inner subventricular zone; **IZ**, intermediate zone; **MZ**, marginal zone; **OFL**, outer fiberlayer; **OSVZ**, outer subventricular zone; **SP**, subplate; **SVZ**, subventricular zone; **VZ**, ventricular zone.) (Adapted, with permission, from Giandomenico and Lancaster 2017.)

B. Section through an organoid generated from human induced pluripotent stem cells. The area between the white lines is enlarged in the micrograph on the right. The section was stained with antibodies to transcription factors (Satb2, Ctip2, and Pax6) selectively expressed in specific layers in human cortex, demonstrating that a layered cortical structure develops in the organoid. (Micrographs reproduced, with permission, from P. Arlotta.)

Mash1 (Figure 46–12A) as well as by the Dlx1 and Dlx2 proteins.

Similarly, the cerebellum contains several different classes of inhibitory neurons (Purkinje, Golgi, basket, and stellate neurons) and two major classes of excitatory neurons (granule neurons and large cerebellar nucleus neurons). These inhibitory and excitatory neurons have different origins; GABAergic neurons derive from the ventricular zone, whereas glutamatergic neurons migrate into the cerebellum from the rhombic lip. The generation of GABAergic and glutamatergic neurons is controlled by two different bHLH transcription factors, Ptf1a for inhibitory and Math-1 for excitatory neurons (Figure 46–12B). These bHLH factors are expressed by neuroepithelial cells but not by mature neurons, implying that differentiation into glutamatergic and GABAergic neurons is initiated prior to neuronal generation.

Transcriptional programs also determine the transmitter phenotype in the peripheral nervous system. For example, BMPs promote noradrenergic neuronal differentiation by inducing the expression of a variety of transcription factors that include the bHLH protein Mash1, the homeodomain protein Phox2, and the zinc finger protein Gata2. In contrast, Runx proteins are determinants of the glutamatergic phenotype of sensory neurons (Figure 46–10).

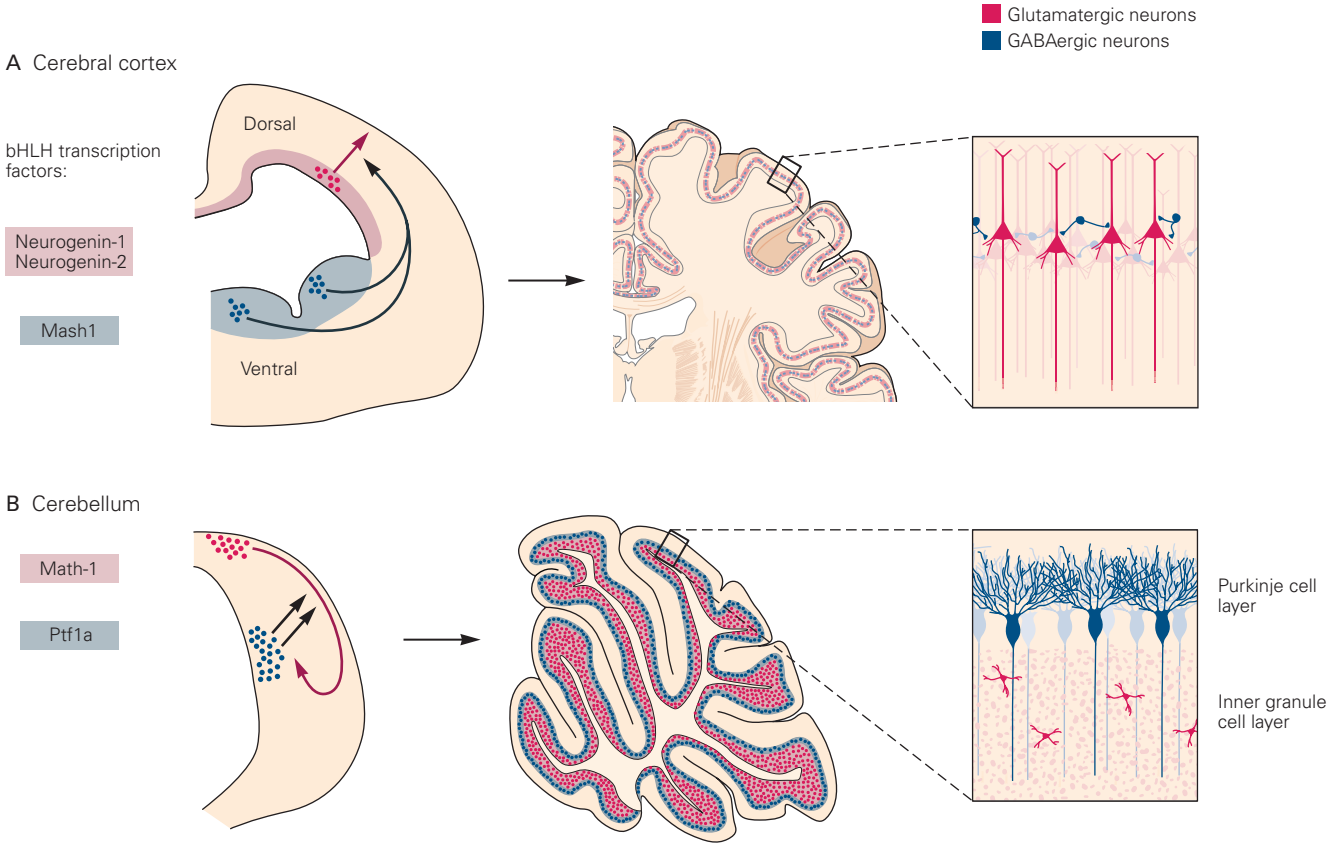

Figure 46–12 The neurotransmitter phenotype of central neurons is controlled by basic helix-loop-helix transcription factors.

A. GABAergic and glutamatergic neurons in the cerebral cortex are generated in different proliferative zones and are specified by different basic helix-loop-helix (**bHLH**) transcription factors. Glutamatergic pyramidal neurons derive from the cortical ventricular zone, and their differentiation depends on the activities of neurogenin-1 and -2. The differentiation of GABAergic interneurons in the ganglionic eminences of the ventral telencephalon depends on the bHLH protein Mash1. These neurons migrate dorsally to supply the cerebral cortex with most of its inhibitory interneurons.

B. GABAergic and glutamatergic neurons in the developing cerebellum also derive from different proliferative zones and are specified by different bHLH transcription factors. Glutamatergic granule cells migrate into the cerebellum from the rhombic lip, settle in the inner granular layer, and are specified by the bHLH protein Math-1. GABAergic Purkinje neurons migrate from the deep cerebellar proliferative zone, settle in the Purkinje cell layer, and are specified by the bHLH protein Ptf1a.

Signals From Synaptic Inputs and Targets Can Influence the Transmitter Phenotypes of Neurons

Because neurotransmitter phenotype is a core neuronal property, it was long thought that transmitter properties were fixed at the earliest stage of neuronal differentiation. This view was challenged by studies showing that the migratory pathway of a neural crest cell exposes the cell to environmental signals that have a critical role in determining its transmitter phenotype.

Most sympathetic neurons use norepinephrine as their primary transmitter. However, those that innervate the exocrine sweat glands in the footpads use acetylcholine, and even these neurons express norepinephrine when they first innervate the sweat glands of the skin. Only after their axons have contacted the sweat glands do they stop synthesizing norepinephrine and start producing acetylcholine.

When the sweat glands from the footpad of a newborn rat are transplanted into a region that is normally innervated by noradrenergic sympathetic neurons, the synaptic neurons acquire cholinergic transmitter properties, indicating that cells of the sweat gland secrete factors that induce cholinergic properties in sympathetic neurons.

Several secreted factors trigger the switch from a noradrenergic to cholinergic phenotype in sympathetic neurons. The sweat gland secretes a cocktail of interleukin-6–like cytokines, notably cardiotrophin-1, leukemia inhibitory factor, and ciliary neurotrophic factor. Several aspects of neuronal metabolism that are linked to transmitter synthesis and release are controlled by these factors. The neurons stop producing the large dense-core granules characteristic of noradrenergic neurons and start making the small electron-translucent vesicles typical of cholinergic neurons (Figure 46–13).

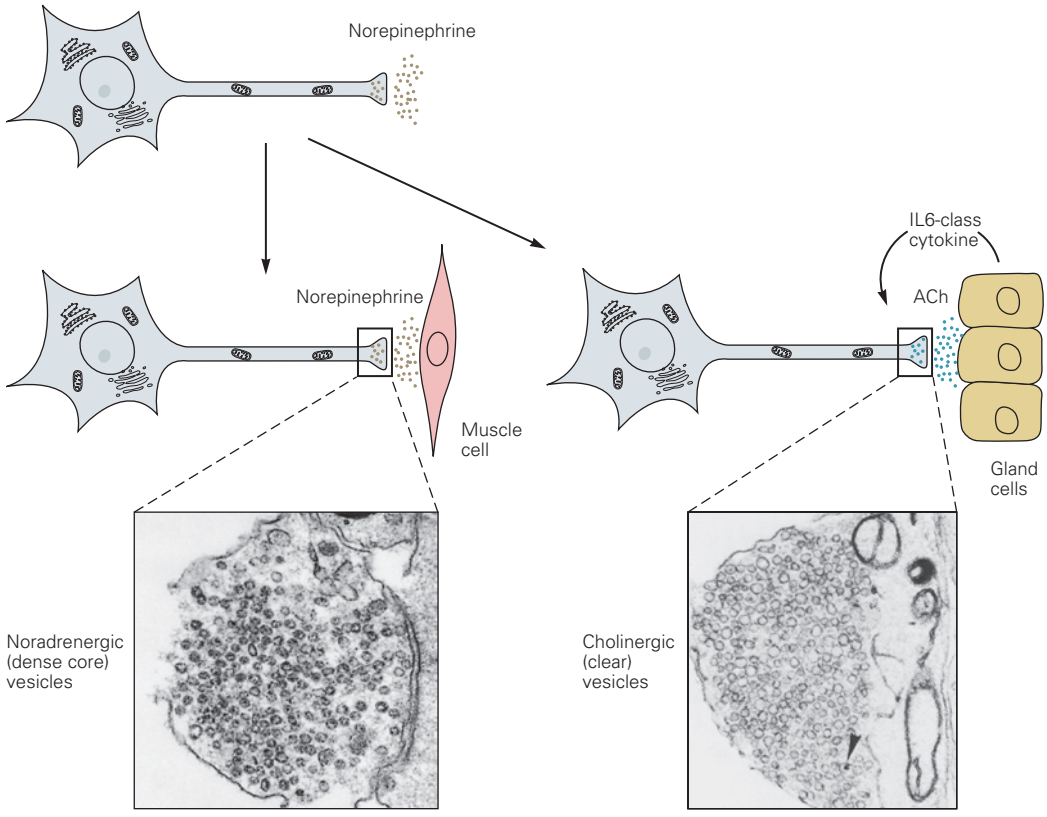

Figure 46–13 The target of sympathetic neurons determines neurotransmitter phenotype. Sympathetic neurons are initially specified with a noradrenergic transmitter phenotype. Most sympathetic neurons, including those that innervate cardiac muscle cells, retain this transmitter phenotype, and their terminals are packed with the dense-core vesicles in which norepinephrine is stored. But the sympathetic neurons that innervate sweat gland targets are induced to switch to a cholinergic transmitter phenotype; their terminals become filled with the small clear vesicles in which acetylcholine (**ACh**) is stored. Sweat gland cells direct the switch by secreting members of the interleukin cytokine family. Several members of this family, including leukemia inhibitory factor and ciliary neurotrophic factor, are potent inducers of cholinergic phenotype in sympathetic neurons grown in cell culture. (Abbreviation: **IL6**, interleukin-6.) (Micrographs reproduced, with permission, from S. Landis.)

More recently, evidence has accumulated that the transmitter phenotype of central neurons can also be influenced by signals including hormones and electrical activity. When the spontaneous activity of embryonic amphibian neurons is increased, some motor neurons can be respecified to synthesize and use the inhibitory neurotransmitter GABA instead of or in addition to acetylcholine. Conversely, when activity is decreased, some inhibitory neurons switch to using the excitatory neurotransmitter glutamate along with or instead of GABA. Postsynaptic partners typically express new receptors that correspond to the transmitter being released onto them. These switches occur without overall respecification of the neuron and are best viewed as homeostatic responses aimed at keeping the overall activity of the system in a narrow range.

Although such transmitter switches in central neurons are likely to occur only rarely under natural conditions, activity-dependent neurotransmitter plasticity may be a more common phenomenon in the adult nervous system. For example, changes in the light cycle where rodents are housed can lead to reciprocal changes in the numbers of neurons that use dopamine and somatostatin as neuromodulators in areas of the brain responsible for maintaining a circadian rhythm. In this and other cases, neurotransmitter switching has measurable consequences on the behavior of the animal, suggesting that this process, along with less drastic synaptic changes discussed in Chapter 49, are employed by the brain as responses to novel environments.

The Survival of a Neuron Is Regulated by Neurotrophic Signals From the Neuron's Target

One of the more surprising findings in developmental neuroscience is that a large fraction of the neurons generated in the embryonic nervous system end up dying later in embryonic development. Equally surprising, we now know that the potential for cell death is preprogrammed in most animal cells, including neurons. Thus, decisions about life and death are aspects of a neuron's fate.

The Neurotrophic Factor Hypothesis Was Confirmed by the Discovery of Nerve Growth Factor

The target of a neuron is a key source of factors essential for the neuron's survival. The critical role of target cells in neuronal survival was discovered in studies of the dorsal root ganglia.

In the 1930s, Samuel Detwiler and Viktor Hamburger discovered that the number of sensory neurons in embryos is increased by transplantation of an additional limb bud into the target field and decreased if the limb target is removed. At the time, these findings were thought to reflect an influence of the limb on the proliferation and subsequent differentiation of sensory neuron precursors. In the 1940s, however, Rita Levi-Montalcini made the startling observation that the death of neurons is not simply a consequence of pathology or experimental manipulation, but rather occurs during the normal program of embryonic development. Levi-Montalcini and Hamburger went on to show that removal of a limb leads to the excessive death of sensory neurons rather than a decrease in their production.

These early discoveries on the life and death of sensory neurons were quickly extended to neurons in the central nervous system. Hamburger found that approximately half of all motor neurons generated in the spinal cord die during embryonic development. Moreover, in experiments similar to those performed on sensory ganglia, Hamburger discovered that motor neuron death could be increased by removing a limb and reduced by adding an additional limb (Figure 46–14A,B). These findings indicate that signals from target cells are critical for the survival of neurons within the central as well as peripheral nervous system. In some cases, manipulating synaptic activity affects the extent of death, perhaps by modulating the types or amount of signals that the target cell produces (Figure 46–14C). We now know that the phenomenon of neuronal overproduction, followed by a phase of neuronal death, occurs in most regions of the vertebrate nervous system.

The early discoveries of Levi-Montalcini and Hamburger laid the foundations for the *neurotrophic factor hypothesis.* The core of this hypothesis is that cells at or near the target of a neuron secrete small amounts of an essential nutrient or trophic factor and that the uptake of this factor by nerve terminals is needed for the survival of the neuron (Figure 46–15). This hypothesis was dramatically confirmed in the 1970s when Levi-Montalcini and Stanley Cohen purified the protein we now know as nerve growth factor (NGF) and showed that this protein is made by target cells and supports the survival of sensory and sympathetic neurons in vitro. Moreover, neutralizing antibodies directed against NGF were found to cause a profound loss of sympathetic and sensory neurons in vivo.

Neurotrophins Are the Best-Studied Neurotrophic Factors

The discovery of NGF prompted a search for additional neurotrophic factors. Today, we know of over a

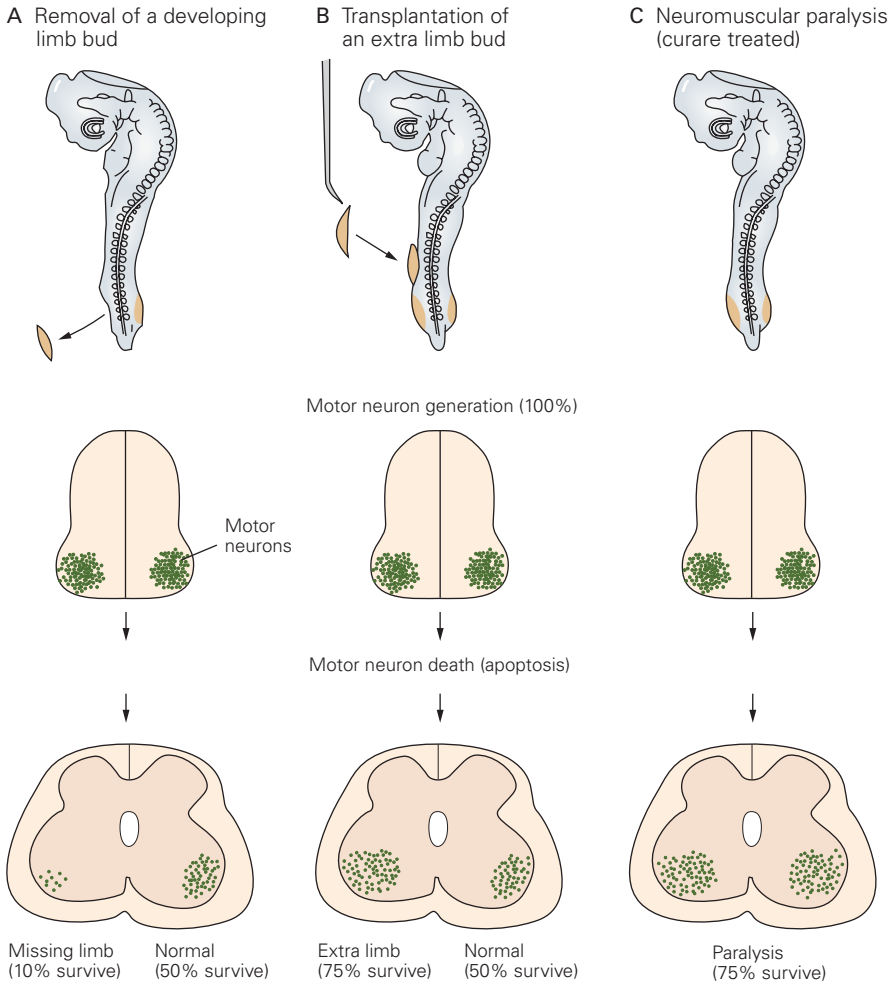

Figure 46–14 **The survival of motor neurons depends on signals provided by their muscle targets.** The role of the muscle target in motor neuron survival was demonstrated by Viktor Hamburger in a classic series of experiments performed on the chick embryo. (Adapted from Purves and Lichtman 1985.)

A. A limb bud was removed from a 2.5-day-old chick embryo soon after the arrival of motor nerves. A section of the lumbar spinal cord 1 week later reveals few surviving motor neurons on the deprived side of the spinal cord. The number of motor neurons on the contralateral side with an intact limb is normal.

B. An extra limb bud was grafted adjacent to a host limb prior to the normal period of motor neuron death. A section of the lumbar spinal cord 2 weeks later shows an increased number of limb motor neurons on the side with the extra limb.

C. Blockade of nerve-muscle activity with the toxin curare, which blocks acetylcholine receptors, rescues many motor neurons that would otherwise die. Curare may act by enhancing the release of trophic factors from inactive muscle.

dozen secreted factors that promote neuronal survival. The best-studied are related to NGF and are called the neurotrophin family.

There are four main neurotrophins: NGF itself, brain-derived neurotrophic factor (BDNF), and neurotrophins-3 and -4 (NT-3 and NT-4). Other classes of proteins that promote neuronal survival include members of the transforming growth factor β family, the interleukin-6–related cytokines, fibroblast growth factors, and even certain inductive signals we encountered earlier (BMPs and hedgehogs). Other neurotrophic factors, notably members of the glial cell line–derived neurotrophic factor (GDNF) family, are responsible for the survival of different types of sensory and sympathetic neurons (Figure 46–16).

Neurotrophins interact with two major classes of receptors, the Trk receptors and p75. Neurotrophins promote cell survival through activation of Trk receptors. The Trk family comprises three membrane-spanning tyrosine kinases named TrkA, TrkB, and TrkC, each of which exists as a dimer (Figure 46–17).

Much is now known about the intracellular signaling pathways activated by binding of neurotrophins to Trks. As with other tyrosine kinase receptors, the binding of neurotrophins to Trk receptors leads to dimerization of the Trk proteins. Dimerization results in phosphorylation of specific tyrosine residues in the activation loop of the kinase domain. This phosphorylation leads to a conformational change in the receptor and to phosphorylation of tyrosine residues that serve as docking sites for adaptor proteins. The adaptors then trigger production of second messengers that both promote the survival of neurons and trigger their maturation. These divergent biological responses involve different intracellular signaling pathways: neuronal differentiation largely via the mitogen-activated protein kinase (MAPK) enzymatic pathways and survival largely via the phosphatidylinositol-3 kinase pathway (Figure 46–18).