5

Neurogenesis

Monica L. Vetter and Richard I. Dorsky

INTRODUCTION

The function of the nervous system is controlled at the most basic level by individual cells—the neurons. In order to generate the enormous diversity of function and connectivity present in the mature nervous system, each neuron must be directed to differentiate at a particular time and place and to adopt a particular phenotype. The process of generating a neuron from a field of neurectodermal cells, known as neurogenesis, is the focus of this chapter. We will largely focus on neurogenesis in the vertebrate nervous system, but when appropriate will use examples from invertebrates to illustrate conserved aspects of nervous system development and in some cases demonstrate molecular mechanisms.

In every vertebrate nervous system, neural precursor cells initially occupy a uniform neuroepithelial sheet. The central nervous system (CNS) arises from a flat neural plate that is patterned along the rostral/caudal (RC) and dorsal/ventral (DV) axes by signals in the embryo beginning during gastrulation (see Chapter 3), while the neural crest and placodes, which are the source for cells of the peripheral nervous system (PNS), arise from the lateral border of this tissue (see Chapter 4). The neural plate eventually rolls (or intercalates in the case of fish) into a neural tube forming a lumen at the center, which defines the ventricular surface of the neural tube. At early stages of development the neural tube consists of proliferating neuroepithelial cells that are multipotent and give rise to all of the major cell populations of the CNS and much of the PNS (see Chapter 2). Throughout development, proliferating neuroepithelial cells remain in contact with the ventricular surface of the neural tube forming a ventricular zone (VZ—see Chapter 2). This zone contains the proliferating cells throughout CNS development, at all rostrocaudal levels of the embryo. As neuroepithelial cells begin the process of differentiation into CNS neurons they detach from the ventricular surface, exit the cell cycle, and migrate away from the VZ to their final location in the developing mantle layer (see Fig. 1A). Neuroepithelial cells also give rise to neural crest cells, which delaminate from the dorsal aspect of the neural tube, migrate away from the neural tube, and differentiate into a variety of cell types, including neurons of the PNS (see Chapter 4).

The cellular process of neurogenesis can be generally considered as a progression from multipotent stem cells to fate-restricted neuronal precursors, through the gradual reduction of potential fates. Once a particular cell fate has been specified, neurons will withdraw from the cell cycle and differentiate. In this chapter we will illustrate the many steps of neurogenesis and provide examples that explain the genetic and molecular mechanisms behind each step. First, cells from the neuroectoderm acquire the competence to become neural, and these stem cells expand to provide the raw material for all subsequent cell generation. In the next step, neural progenitors are produced by asymmetric divisions of stem cells, lose the ability to self-renew, and begin to be restricted in potential. Cell number is tightly controlled at these early stages through regulation of both proliferation and survival of stem cells and progenitors. Third, neural progenitors express genes that promote differentiation, while negative regulators constrain the number of neurons that are generated at any given place and time. The fourth step of neurogenesis is the irreversible decision to leave the cell cycle and form a neuron. Fifth, neural precursors migrate to their final position in the nervous system and differentiate. Finally, neurons mature and adopt a particular phenotype by activating gene programs that direct their ultimate differentiation into functioning neurons. Many different subtypes of neurons exist in the mature nervous system. During development it is essential that the generation of these different classes of neurons be carefully orchestrated so that functionally integrated neuronal structures can assemble.

The two main processes that contribute to the generation of neuronal diversity are spatial patterning and temporal regulation of birthdates. Through the combination of these two events, each neural progenitor has a unique positional identity and history by virtue of being exposed to a different combination of inductive factors. This ultimately results in neural progenitors expressing a distinct combination of transcription factors that will regulate their differentiation into specific neuronal subtypes. In some cases the phenotype of a differentiating neuron can also be influenced as it migrates to its final position, or after innervation

Monica L. Vetter and Richard I. Dorsky • Department of Neurobiology and Anatomy, University of Utah, SOM, Salt Lake City, UT 84132.

Developmental Neurobiology, 4th ed., edited by Mahendra S. Rao and Marcus Jacobson. Kluwer Academic / Plenum Publishers, New York, 2005.

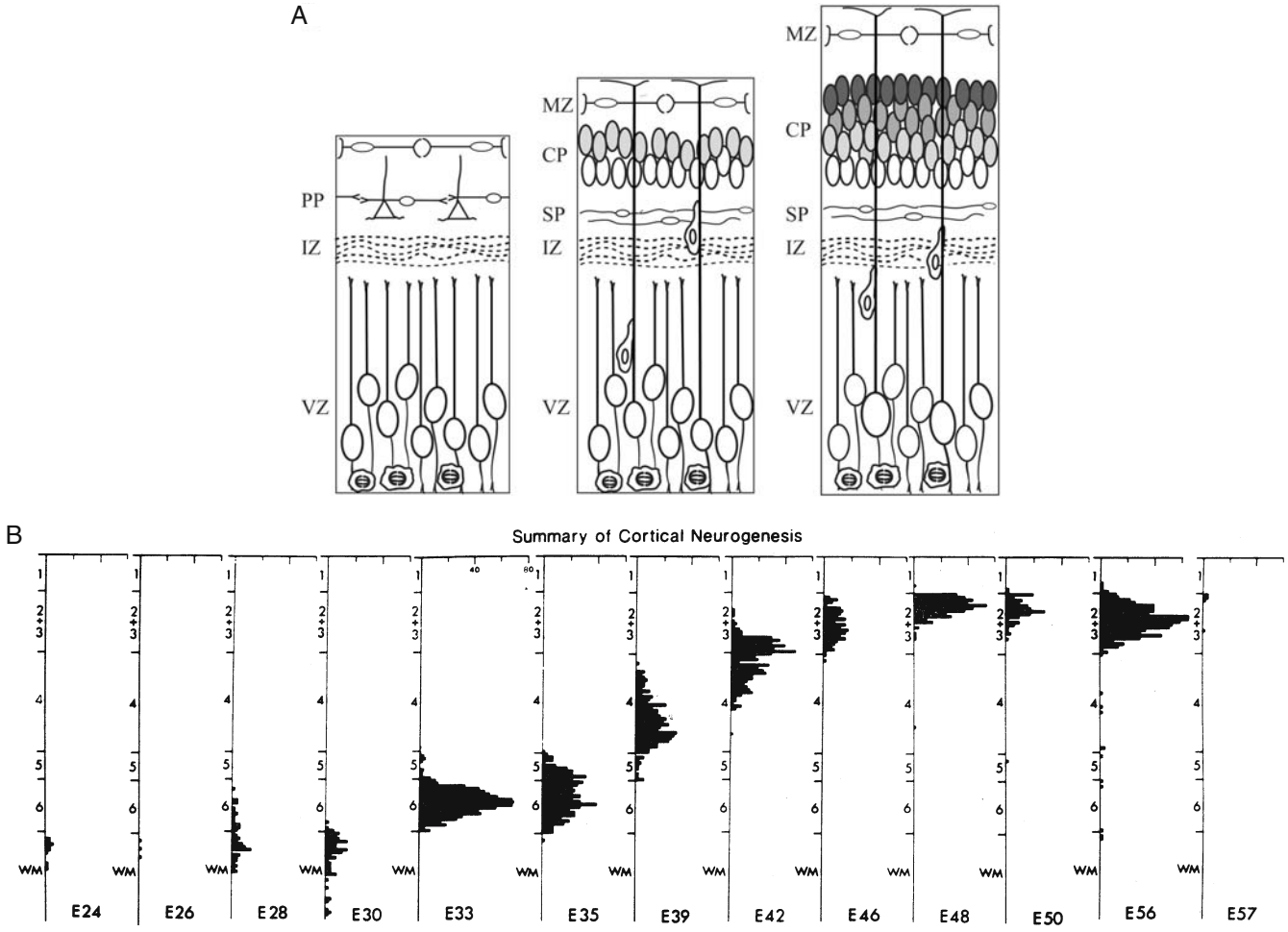

FIGURE 1. (A) Development of the cerebral cortex. The ventricular zone (VZ) contains proliferating progenitors that divide at the ventricular surface. The first neurons to differentiate are those forming the preplate (PP), which is separated from the VZ by PP axons and incoming thalamic axons in the intermediate zone (IZ). As development progresses the cortical plate (CP) forms from neurons which migrate out from the VZ along radial glial fibers, separating the PP into the subplate (SP) and superficial marginal zone (MZ). Within the CP, deep layer neurons are generated first and later-born neurons migrate past the early-born neurons to populate more superficial layers (dark grey). Ultimately, the SP neurons and VZ disappear and the MZ becomes layer I of the mature cortex. The CP neurons develop into the remaining cortical layers (II–VI) and overlay the white matter. Figure generated by Diana Lim. (B) Cortical neurons are born in an inside-out sequence. Each histogram shows the relative depth distribution of heavily labeled neurons in the developing visual cortex of the cat resulting from a single injection of [^{3}H]thymidine given at the embryonic age shown underneath. Neurons of different cortical layers are generated in an inside-out sequence between E30 and E57. Modified from M.B. Luskin and C.J. Shatz, 1985, *J. Comp. Neurol.* 242:611–631.

of its target tissue. We will now consider in detail each of these steps in the process of neurogenesis, beginning with an overview of histogenesis, the cellular process of differentiation, in different parts of the developing nervous system.

HISTOGENESIS IN THE VERTEBRATE NERVOUS SYSTEM

Birthdating, Transplantation, and Lineage Analysis

The vertebrate nervous system is a highly organized tissue and its cellular organization is critical for its proper function. In many parts of the nervous system the tissue is laminated; that is, neurons with similar structural and functional properties are organized into discrete layers. In other places, neurons assemble into nuclei or ganglia rather than layers. How are these patterns of tissue organization established? Historically, several techniques have been important for defining how neurons are generated and become organized within specific domains of the developing nervous system. The birthdating technique, developed by Richard Sidman in the late 1950s, can be used to label groups of neurons as they are born and then track them to their final position (Sidman *et al*., 1959). This method involves labeling proliferating precursor cells within an embryo by pulsing with tritium-labeled thymidine, which incorporates into the DNA during replication. If the cell continues to divide then this label

becomes diluted through subsequent rounds of DNA synthesis. However, if a cell becomes labeled during its final division and subsequently differentiates, then that cell remains heavily labeled and can be detected by autoradiography of histological sections. The "birthdate" of a cell is defined as the time when it undergoes its final division, and this can be assessed by pulsing with tritiated thymidine at various times in development and determining when that type of cell becomes heavily labeled. In addition, by analyzing the location of heavily labeled cells at progressively later times following a pulse of tritiated thymidine, it is possible to track the position of cells born at a particular time as they migrate to their final position.

The fate of cells can also be followed by transplanting cells from one species into another then using specific markers or cellular features to distinguish donor cells from host. For example, Nicole Le Dourain used a heterochromatin marker in the nuclei of quail cells to track them after transplantation into chick embryos (Le Douarin, 1973, 1982). This approach has not only been valuable for tracking the migratory pathways of cells, particularly those derived from the neural crest, but has also made it possible to transplant cells into new environments to determine their developmental potential.

The third technique, called lineage analysis, made it possible to track all of the progeny from a single precursor cell and determine their phenotypes and their ultimate resting position. One approach to lineage analysis is to intracellularly inject a tracer such as a fluorescent dye or horseradish peroxidase that would be passed on to the progeny of that cell (Fig. 2; Weisblat *et al*., 1978). This approach can be problematic since multiple rounds of cell division can dilute the tracer, so it is not always a reliable marker of lineage. Alternatively, retroviruses carrying a reporter gene can be used to stably label cells and their progeny (Cepko, 1988). Small amounts of retroviruses are injected so that only a few proliferating progenitor cells become infected and their progeny can be followed. One problem with this approach is that it is difficult to determine whether all labeled progeny in a given domain were derived from a single infected progenitor. To address this concern, libraries of retroviruses have been used carrying large numbers of individual tags that can be distinguished by amplifying specific tag sequences using the polymerase chain reaction (PCR; Walsh and Cepko, 1992). A single retrovirus will infect a progenitor and the labeled progeny will all carry the same tag, arguing for clonal origin.

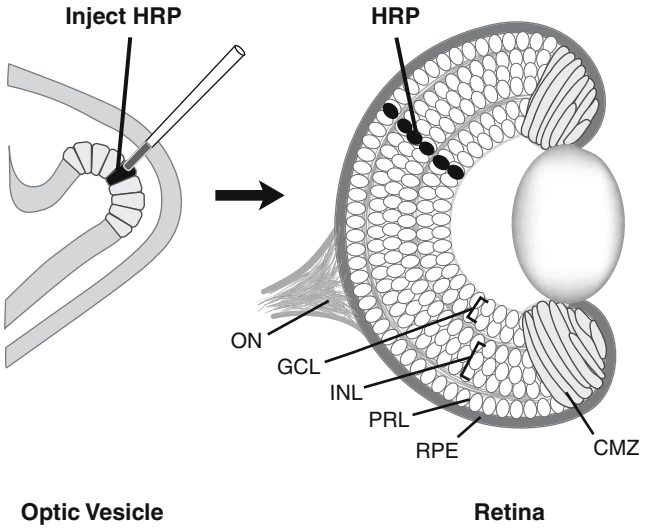

FIGURE 2. Retinal progenitors are multipotent. Injection of HRP, a lineage tracer, into a single retinal progenitor at the optic vesicle stage in *Xenopus laevis* reveals that a single progenitor can generate multiple retinal cell types that span the layers of the mature retina (Holt *et al*., 1988). HRP, horseradish peroxidase; ON, optic nerve; GCL, ganglion cell layer; INL, inner nuclear layer; PRL, photoreceptor layer; RPE, retinal pigment epithelium; CMZ, ciliary marginal zone. Figure generated by Diana Lim.

Together these approaches have revealed a few general principles in nervous system development. First, the birthdate of a neuron is an important predictor of cell fate. In a given region, neurons born at a certain time generally adopt similar fates. Second, newborn neurons often migrate a considerable distance from their site of origin to their final resting place. Finally, within a given region of the nervous system, neurons of similar phenotype and birthdate cluster together in discrete layers, nuclei, or ganglia. We will consider several examples of histogenesis in the developing vertebrate nervous system to illustrate these points.

Cerebral Cortex

The mature cerebral cortex is a beautiful example of a laminated neuronal tissue. The mammalian neocortex consists of six layers that can be distinguished histologically based upon the morphology and density of neurons within each layer. This also reflects distinct functions for the neurons in each layer. Layer I is closest to the pial surface and contains relatively few neurons. Neurons in layers II/III provide connections between different cortical areas, while layer IV neurons receive inputs from subcortical structures such as the thalamus. Layer V and VI neurons send projections to subcortical structures, such as thalamus, brainstem, and spinal cord. The thickness of these layers varies depending upon whether a given cortical region serves largely sensory, motor, or association functions. This precise laminar organization is important for proper functioning of the neocortex. Developmental disorders that result in disruption of neurogenesis and lamination of the cortex are associated with severe mental retardation and epilepsy.

The cerebral cortex begins as a single layer of proliferating neuroepithelial cells in the walls of the telencephalon. At some point these neuroepithelial cells begin to divide asymmetrically generating first neurons and later glia. Birthdating studies have revealed a very tight correlation between birth order of neurons and their final laminar position (Angevine and Sidman, 1961). In the mammalian cortex, the earliest generated neurons migrate away from the VZ and form a layer of cells beneath the pial surface known as the preplate (Fig. 1A). Later-generated neurons then migrate into the preplate to form the cortical plate, thus splitting the preplate into a superficial marginal zone (future layer I) and a deeper zone called the intermediate zone that contains subplate neurons and incoming axons. Thus both

the marginal and intermediate zones contain neurons that were generated earliest. The marginal zone neurons include Cajal-Retzius cells, which provide important signals for later-born neurons as they migrate out and establish the cortical layers (see Chapter 8). The subplate neurons in the intermediate zone serve a transient developmental role as guideposts for incoming thalamic axons preparing to innervate the cortical layers.

Within the developing cortical plate, tritiated thymidine labeling reveals a very orderly pattern of generation, migration, and assembly of neurons in tangential strata (Fig. 1B; Angevine and Sidman, 1961). The emerging cortical layers are established in an inside-out sequence such that deep layer neurons are born first followed progressively by neurons that will migrate radially past the deep layer neurons to occupy more superficial layers (Fig. 1A). Thus, pulsing with thymidine at early stages of development results in labeling of neurons in deeper layers of the cortical plate, while pulsing at later stages of development results in labeling of more superficial layers. The older deep layer neurons have already begun to differentiate and send out axons as the later-born neurons migrate past them to populate the more superficial layers. In addition, there are spatial gradients across the cortex with respect to the timing of neurogenesis in different cortical regions. Even in three-layered allocortex, such as the hippocampus, deep neurons are generated before superficial neurons and the younger neurons migrate through previously formed layers to generate more superficial layers (Angevine, 1965).

In general, excitatory projection neurons follow this pattern of genesis and migration (Tan *et al*., 1998). They are generated from progenitors in the VZ and then migrate radially to populate the emerging cortical layers in radial columns, although there is also evidence for non-radial tangential migration of developing cortical neurons (O'Rourke *et al*., 1995, 1997; see Chapter 8). However, lineage analysis and studies of neuronal migration have revealed that most local circuit GABAergic inhibitory interneurons are generated from a distinct population of progenitors in subcortical ventral forebrain regions (Tan *et al*., 1998). These interneurons are born in the VZ of the lateral and medial ganglionic eminences, then migrate dorsally and disperse through the cortical layers (Anderson *et al*., 1997; Lavdas *et al*., 1999; Parnavelas *et al*., 2000).

At early stages of cortical development, neurons are generated from progenitors in the VZ, although the VZ diminishes as the cortex develops. At later stages of vertebrate development a second zone of proliferating cells known as the subventricular zone (SVZ) forms between the VZ and the intermediate zone. As the VZ disappears, the SVZ continues to proliferate and generate cortical neurons, as well as most of the glial cells in the cortex. The SVZ also gives rise to neurons that will migrate to the olfactory bulb along a specific migratory path known as the rostral migratory stream (Lois and Alvarez-Buylla, 1994). Although the SVZ also diminishes as development progresses, there is good evidence that the SVZ retains its capacity to generate new cells in the adult (Lois and Alvarez-Buylla, 1993), a topic that will be discussed in more detail later.

Retina

Like the cerebral cortex, the vertebrate retina is a laminated CNS structure consisting of three major cellular layers. The outermost layer closest to the non-neural retinal pigment epithelium is the photoreceptor layer and contains rod and cone photoreceptors. The middle layer, called the inner nuclear layer (INL), contains several classes of interneurons such as horizontal cells, bipolar cells, and amacrine cells. The innermost layer closest to the vitreal surface is the retinal ganglion cell layer, which consists of retinal ganglion cells, the projection neurons of the retina, and in some species considerable numbers of displaced amacrine cells. There is also one major type of glial cell in the retina, the Müller glial cell, which spans the width of the retina with the cell body being localized to the INL.

The retina begins as a single cell-wide epithelial sheet, and progenitors are attached to both the outer (ventricular) and inner limiting membranes, which are composed of neuroepithelial and eventually glial endfeet. As they proceed through the cell cycle, progenitor nuclei migrate from the outer surface (M-phase) to the inner surface (S-phase) in a process termed interkinetic migration (see Chapter 2). As progenitors continue to proliferate, the retinal thickness expands and dividing cells are split into inner and outer neuroblastic layers. The inner neuroblastic layer will eventually differentiate into ganglion, amacrine, and Müller cells, while the outer neuroblastic layer produces photoreceptor, horizontal, and bipolar cells. While there is no true "radial migration" of neural precursor cells in the retina, cells do detach from the retinal surfaces and move to their ultimate positions. As rod, bipolar, and Müller cells differentiate, neurons derived from the same region of neuroepithelium remain spatially associated. In contrast, cone, ganglion, horizontal, and amacrine cells undergo extensive tangential migration (Fekete *et al*., 1994; Reese *et al*., 1995).

Cell birthdating studies using the methods described previously have shown a generally conserved order of genesis for retinal cell types across all vertebrate species (Cepko *et al*., 1996). Ganglion cells, the projection neurons of the retina, are the first cell type born, shortly followed by horizontal and amacrine interneurons, and cone photoreceptors. At the end of histogenesis, late-born cell types include rod photoreceptors, bipolar cells, and Müller glia. In rapidly developing vertebrates such as *Xenopus*, there is considerable overlap between the birthdates of these cell types, but the general order is preserved (Holt *et al*., 1988). Importantly, this order suggests that some factor, either internal or external to the retinal progenitors, biases them toward particular fates at different times during development. Although cell fate in the retina is partially determined by temporal order of histogenesis, birth order does not correlate with laminar position, which is unlike the cerebral cortex. Instead, as progenitors withdraw from the cell cycle and differentiate, they migrate to the appropriate position for their function.

Interestingly, retinal histogenesis continues throughout the life of the animal in fish and frogs. As the eye continues to grow in these animals, new cells are added to the periphery from a structure called the ciliary marginal zone (CMZ) (see Fig. 2).

The CMZ has been studied as a model of retinal cell-fate determination because all the mature cell types are generated from this small region, and at any given time, all stages of progenitor development can be observed (Perron *et al*., 1998). Furthermore, these characteristics of the CMZ suggest that extracellular signals influencing cell fate must be supplied very locally.

Spinal Cord

The spinal cord has become an important model system for studying neural cell-fate specification because it contains populations of anatomically and molecularly identifiable motoneurons and interneurons and a transient population of sensory neurons. In addition, the spinal cord is a relatively simple CNS structure in which histogenesis follows the same general rules as other regions of the nervous system. Proliferation takes place in the VZ, which, as in the cortex and retina, begins as a single cell-wide neuroepithelium. Progenitors undergo interkinetic nuclear migration then detach from the ventricular surface and migrate laterally through an intermediate zone into a mantle zone where they differentiate. In addition to radial migration, some differentiating precursors migrate tangentially in the intermediate zone, along dorsoventral and rostrocaudal pathways (Leber and Sanes, 1995). Therefore the final position of differentiated spinal neurons often does not correspond to the region from which they were generated.

The general order of histogenesis in the spinal cord is the same as in the brain—neurons are generated first, followed by astrocytes and oligodendrocytes. Within the neuronal population, there is also a conserved order of birth. Ventral motoneurons are born first, followed by more dorsal interneurons (Nornes and Carry, 1978). Single progenitors can give rise to multiple subtypes of neurons, and some produce both neurons and glia. As in the retina, it appears that both the timing and spatial localization of differentiation play important roles in ultimate cell fate. Particular types of neurons and glia arise from different dorsoventral positions in the VZ. In addition, progenitor fate appears to be restricted over time, to the point where some glial and neural-restricted precursors have been identified by clonal analysis both in culture and *in vivo* (Mayer-Proschel *et al*., 1997; Rao *et al*., 1998).

Different Classes of PNS Neurons Have Distinct Birthdates

Even in the PNS, different subtypes of neurons are born at different times and aggregate into discrete domains. For example, neurons in the dorsal root ganglia (DRG) are derived from neural crest precursor cells that have migrated away from the neural tube and aggregated into ganglia (see Chapter 4). Within the developing DRG, precursor cells proliferate then ultimately stop dividing and differentiate. The DRG contains several different classes of sensory neurons, such as proprioceptive and cutaneous neurons, which are born in an overlapping sequence (Carr and Simpson, 1978). These different types of DRG neurons then partially segregate within the DRG. For example, in chick, most proprioceptive neurons are born early and occupy the ventral half of the ganglia, while cutaneous neurons are, for the most part, born later than the proprioceptive neurons and are more broadly distributed within the ganglia, including the dorsal domain (Carr and Simpson, 1978; Henrique *et al*., 1995). There is now evidence that early markers can distinguish these cell populations even before their axons reach their targets, suggesting that their fates are determined early (Guan *et al*., 2003).

Conserved Role of Timing in Neurogenesis

In all these different regions of the vertebrate nervous system there is evidence of a strong link between birthdate and neuronal phenotype, suggesting that there is temporal regulation of the neuronal cell-fate decision. In fact, this appears to be a conserved feature of neurogenesis across animal phyla. We can use this conservation to help study the process of neurogenesis in simpler invertebrate organisms that are amenable to genetic manipulation. The most fruitful of these studies have taken place in *Drosophila*, where precise examination of neurogenesis has been undertaken throughout development. In the *Drosophila* embryonic CNS, precise numbers of neurons are generated from single neuroblasts in a defined temporal sequence. Individual neuroblasts arise from the ectoderm then divide to produce a series of ganglion mother cells (GMCs; see Fig. 3). These cells then divide to produce neuronal and glial siblings that undergo terminal differentiation. GMCs are produced sequentially and each successive GMC generates different progeny. If an individual GMC is ablated, its fate is skipped entirely and the next GMC goes on to produce daughters appropriate for its time of generation (Doe and Smouse, 1990). Thus there is a tight link between the birthdate of a GMC and the phenotype of the cells that it generates.

We will now step back and consider how neurogenesis is regulated, highlighting examples from both vertebrate and invertebrate nervous system development.

NEUROEPITHELIAL CELLS ARE MULTIPOTENT AND HAVE POSITIONAL IDENTITY

The vertebrate neural tube is initially formed of highly proliferative neuroepithelial cells that when isolated and placed in culture exhibit properties characteristic of neural stem cells: They are capable of long-term self-renewal and can generate the major cell types of the nervous system—neurons, astrocytes, and oligodendrocytes (see Chapter 2). In addition, infection of these early stem cells with retroviral lineage tracers *in vivo* shows that a single progenitor cell can give rise to all three major cell types (Kalyani and Rao, 1998). These neuroepithelial cells have long processes that span the width of the early neural tube; however, cell division occurs at the ventricular surface (see Chapter 2). Neuroepithelial cells initially divide symmetrically expanding the pool of early neural stem cells. In symmetric divisions

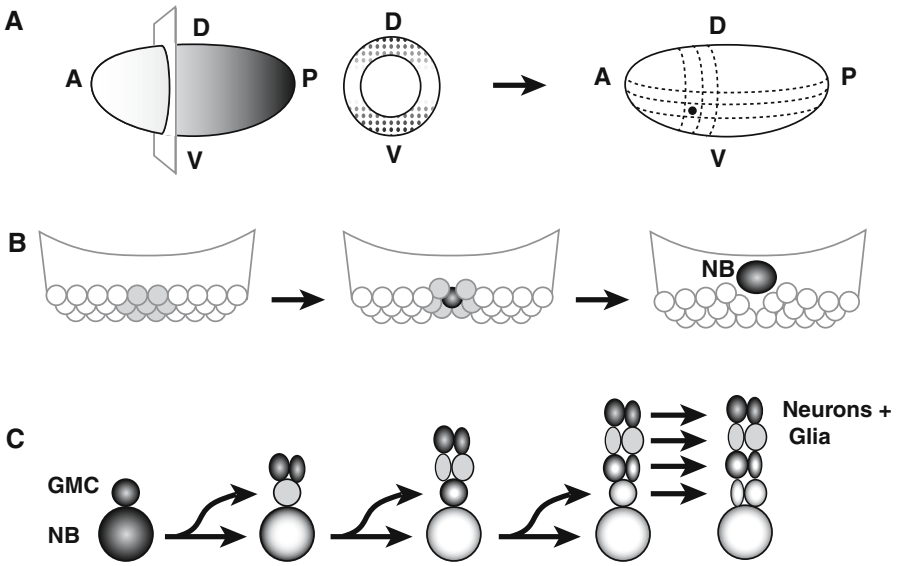

FIGURE 3. Neuroblast development in the *Drosophila* CNS. (A) Gradients of signaling molecules pattern the early *Drosophila* embryo along the anterior–posterior (AP) and dorsal/ventral (DV) axes. The embryo is thus subdivided by the expression of segment polarity genes (vertical stripes) and columnar genes (horizontal stripes), and each neuroblast within these segments (black dot—only one shown) has a positional identity that determines the phenotype of the cells that it generates. (B) Within the neuroectoderm a neuroblast (NB) is selected from a cluster of cells (light grey) through a process of lateral inhibition (see text) and delaminates from the ectoderm. All cells within the cluster (light grey) initially express equivalent levels of proneural genes. As the neuroblast is selected it expresses elevated levels of proneural genes, while the surrounding cells downregulate proneural gene expression and assume a non-neural ectodermal fate. (C) The neuroblast undergoes a series of divisions to generate ganglion mother cells (GMCs) which then divide and differentiate into neurons and glia of the ventral nerve cord. Figure generated by Diana Lim.

the plane of cell division is perpendicular to the ventricular surface generating two identical daughters (Chenn and McConnell, 1995). This mode of division is important for self-renewal and is prominent during the early expansion phase of neuronal development.

Coincident with neural induction, the nervous system becomes patterned along the RC and DV axes in response to gradients of signaling molecules from neighboring tissues (see Chapter 3). As a result, neuroepithelial cells at the earliest stages of development already have a positional identity and express genes appropriate for their region of origin even when isolated and grown in culture. This positional identity influences the types of neurons that arise from precursors in different parts of the nervous system. For example, neuroepithelial cells isolated from spinal cord can generate the complement of neuronal cell types appropriate for spinal levels (Kalyani *et al*., 1997, 1998), while basal forebrain stem cells generate GABAergic interneurons similar to those that normally populate the cerebral cortex (He *et al*., 2001). DV position is also important. For example, within the developing spinal cord, progenitors respond to gradients of signaling molecules, such as Sonic hedgehog (Shh) ventrally and BMPs dorsally, that define DV position within the spinal cord. These progenitors then have a unique positional identity that allows them to generate the appropriate types of neurons for that position in the spinal cord, such as ventral motoneurons and dorsal sensory interneurons (Lee and Pfaff, 2001).

As in vertebrates, positional identity is also a critical factor in insect nervous system development, arguing that this is an evolutionarily conserved mechanism for generating regional diversity in the nervous system. During insect CNS development, neuroblasts arise at segmentally repeated positions in the ventral neurogenic region of the embryo in a precise spatiotemporal pattern. Within each hemisegment, around 30 neuroblasts delaminate from the epithelium and begin a series of cell divisions, generating first ganglion mother cells then post-mitotic neurons (Fig. 3). Neuroblasts in different positions within the hemisegment have distinct identities and generate a specific complement of neuronal and glial cell types. The gap and pair-rule genes act prior to neurogenesis to subdivide the embryo into segments along the anterior–posterior (AP) axis (Akam, 1987). Subsequently segment polarity genes, such as wingless (*wg*) and sonic hedgehog (*shh*), pattern the segments and have an important influence on the formation and identity of neuroblasts within a segment (Bhat, 1999). In addition, the dorsal–ventral position of neuroblasts is defined by signaling through NF-κB, BMP, and EGF pathways, which creates DV subdivisions of gene expression within the neuroectoderm (von Ohlen and Doe, 2000). Thus, the combination of AP and DV positional information provides each neuroblast in *Drosophila* with a positional identity and allows it to generate a unique complement of post-mitotic cell types appropriate for that position in the embryo.

NEURAL PROGENITORS ARE MULTIPOTENT BUT BECOME RESTRICTED IN COMPETENCE

Together with positional identity of the progenitors, the temporal birth order of post-mitotic cells from these progenitors

is also a critical variable in determining the ultimate phenotype of the cells that result. In a given region of the vertebrate CNS neurons are generated first, followed by astrocytes then oligodendrocytes. This is also true if neural stem cells are isolated and grown in culture, although this can be influenced by addition of growth factors or other signaling molecules (Qian *et al*., 2000). As development proceeds neuroepithelial cells begin to undergo asymmetric divisions, first generating progenitors for neurons, then for glia in a stage-dependent manner. When placed in culture, these progenitors have a limited capacity for self-renewal and are restricted in their potential, giving rise to a much more limited complement of cell types than the neuroepithelial stem cells (Rao, 1999). Thus, more restricted progenitors can divide to generate neurons that will exit the cell cycle, begin to differentiate, and migrate to their final position.

We know that in each region of the developing nervous system cells are born in a general order, but where do the individual cell types come from? More specifically, are there separate populations of progenitors that produce early and late neuronal cell types, or do they arise from a common pool? The fate of progenitor cells has been examined through a number of lineage-tracing methods, including direct label injection and retroviral infection. The results of these studies confirm that in many parts of the developing nervous system, progenitors are multipotent. For example, in the developing cerebral cortex, progenitor cells are multipotent, giving rise to clones of cells that will populate multiple cortical layers (Walsh and Cepko, 1988). At any given time in development cortical progenitors are biased towards generating cells of specific laminar fates. Deep layer neurons are generated early, while neurons in more superficial layers are generated later (Angevine and Sidman, 1961). Progenitors from older animals normally dedicated to making superficial layer neurons do not make early-born deep layer neurons, even when transplanted back into a younger environment; thus, their competence appears to be restricted over developmental time (Frantz and McConnell, 1996). However, progenitors isolated from the VZ at early stages of development can be transplanted into older animals, and these cells, if transplanted prior to their final division, will respond to their new environment and generate late-born cells appropriate for the later stage of development (McConnell, 1988; McConnell and Kaznowski, 1991). Thus, early cortical progenitors are competent to make both early and late cell types, while later progenitors appear to be restricted in their competence.

In the developing retina, individual retinal progenitors have the ability to produce many different combinations of retinal cells, including neurons and Müller glia (Fig. 2). Lineage analysis has revealed no predictable pattern to the cell composition of retinal clones, ruling out the idea of dedicated progenitors for specific neurons or combinations of neurons (Turner and Cepko, 1987; Holt *et al*., 1988; Turner *et al*., 1990). In many cases progenitors remain multipotent up until their final division generating two nonidentical daughters. Although retinal progenitors are multipotent, at any given stage of development they appear to be limited in their competence and generate only the subset of retinal cell types appropriate for that stage of development (Belliveau and Cepko, 1999; Belliveau *et al*., 2000). This competence appears to change over developmental time so that early retinal progenitors are biased toward making early-born cell types, such as retinal ganglion cells, while later progenitors are biased toward producing later-born fates, such as rod photoreceptors and Müller glia (Livesey and Cepko, 2001). An extreme case of this restriction occurs in the mature fish retina, where a population of dividing precursor cells generates only rods (Raymond and Rivlin, 1987).

Unlike in the cortex, retinal progenitors do not appear to change their intrinsic competence in response to new environments and appear to be restricted to a limited repertoire of fates at different times during development. For example, early progenitors grown in culture continue to generate retinal ganglion cells, an early-born cell type, even when cultured in the presence of older cells (Austin *et al*., 1995). The mechanisms underlying changes in progenitor competence, both in the retina and cerebral cortex, remain to be defined. Although progenitors in many parts of the nervous system are multipotent, in a given region at any one time progenitors are not necessarily a uniform population. There is now good molecular evidence for progenitor diversity in the developing retina and cortex, and this may ultimately contribute to neuronal subtype diversity in the nervous system (Livesey and Cepko, 2001; Nieto *et al*., 2001).

Up to this point, we have described cellular aspects of neuron formation, including the physical development of nervous system structures, and cellular histogenesis. We have also shown that progenitor cells are initially multipotent and become progressively restricted to a limited number of fates due to positional cues from their environment. Next, we will discuss the intrinsic and extrinsic molecular mechanisms by which these cells are driven down the pathway of neurogenesis.

THE PRONEURAL GENES

Like many developmental events, neurogenesis is regulated by a balance between positive regulators that promote neural competence or neuronal differentiation and negative regulators that constrain when and where differentiation occurs. There is evidence that these fundamental mechanisms, although they may vary in detail, are largely conserved during nervous system development of all animals. Subsets of cells within the neural ectoderm are selected to become neural precursors, which will then divide and differentiate to form post-mitotic neurons. How are these neural precursors specified?

Neurogenesis absolutely requires the function of proneural genes, which encode basic helix-loop-helix (bHLH) transcription factors (Bertrand *et al*., 2002). The basic domain in this family of proteins mediates DNA binding to specific DNA sequences known as E boxes (CANNTG), while the helix-loop-helix motif allows heterodimerization with ubiquitously expressed bHLH partners or E proteins (Fig. 4A; Murre *et al*., 1989a, b). Proneural bHLH genes were first described in *Drosophila* and include genes of the *achaete-scute* complex (*achaete, scute, lethal of scute*, and *asense*) and atonal-related genes (*atonal, amos*, and *cato*), which regulate the development of different classes of neurons in the fly PNS and CNS (Bertrand *et al*., 2002). Multiple proneural bHLH

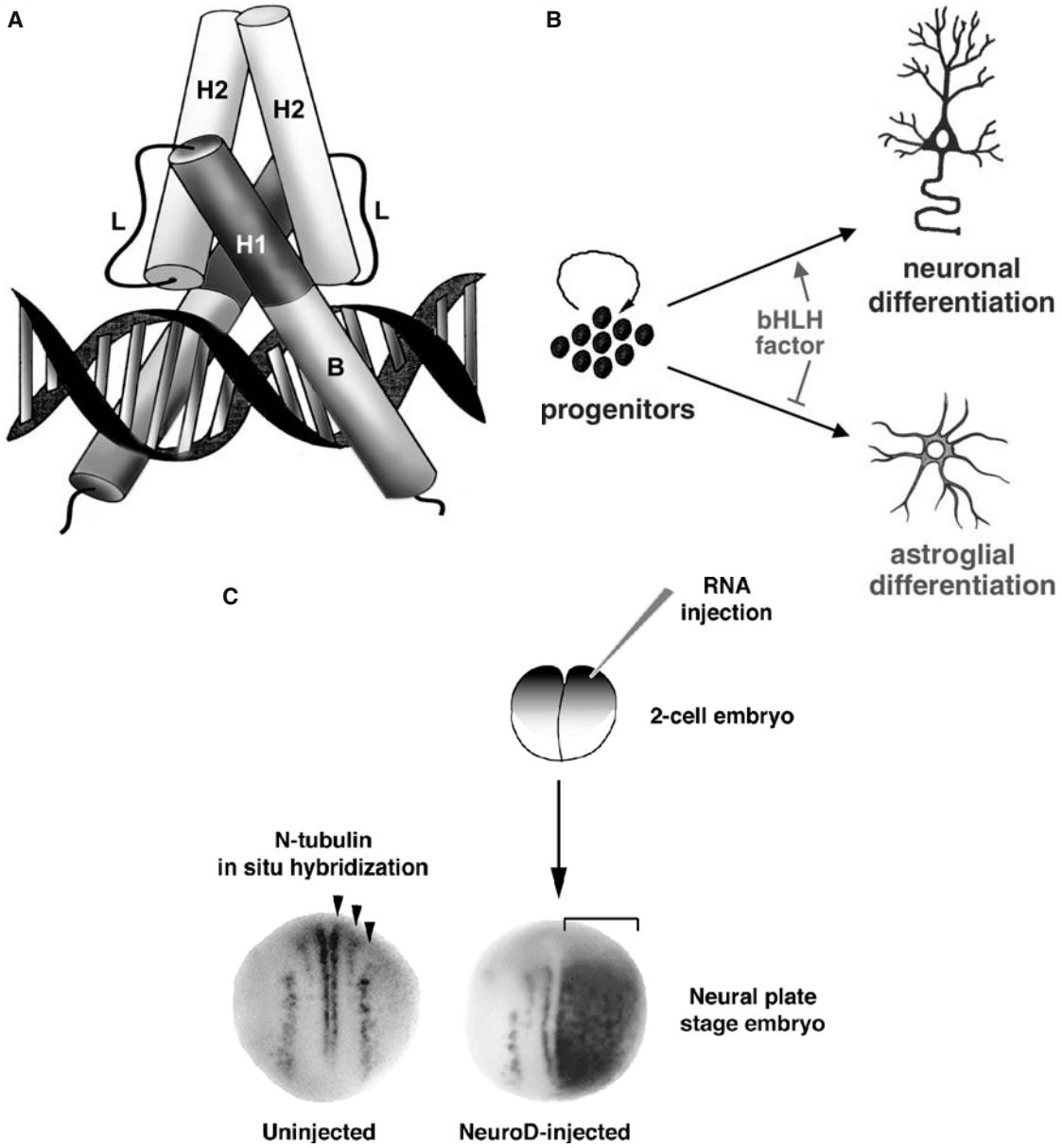

FIGURE 4. (A) Proneural genes encode basic helix-loop-helix (bHLH) transcription factors. The basic domain (B) mediates DNA binding. Helix 1 (H1) and helix 2 (H2) are joined by a loop (L) and mediate dimerization. Figure generated by Diana Lim. (B) Vertebrate proneural bHLH factors act in progenitors to promote the neuronal fate and suppress astroglial fate. (C) Three stripes of primary neurons (arrowheads) develop on either side of the midline in the neural plate of *Xenopus* embryos, as revealed by *in situ* hybridization for the neuronal marker N-tubulin (uninjected). Overexpression of NeuroD by RNA injection into a two-cell stage *Xenopus* embryo promotes ectopic neurogenesis throughout the ectoderm on the injected side (square bracket), showing that NeuroD is sufficient to convert ectodermal cells to a neuronal fate (Lee *et al*., 1995).

genes have been identified in vertebrates and are expressed in distinct domains within the developing CNS. These can be classified into subfamilies based upon their homology to the *Drosophila* proneural genes. One vertebrate subfamily is most closely related to genes of the *achaete-scute* complex in *Drosophila* and includes genes such as *Mash1* (Guillemot and Joyner, 1993). The other subfamily shows stronger homology to *Drosophila atonal* and includes the *Ath* genes, *neurogenins* and *NeuroD*-related genes (Bertrand *et al*., 2002). As in *Drosophila*, different vertebrate proneural bHLH proteins are required for the development of different subpopulations of neurons, and in some cases act redundantly. For example, mice mutant for *neurogenin 1* (*ngn1*) or *ngn2* fail to develop complementary sets of cranial sensory ganglia, while mice mutant for both *ngn1* and *ngn2* lack both these populations of neurons and additionally lack neurons in the ventral spinal cord and DRG (Fode *et al*., 1998; Ma *et al*., 1998, 1999). During vertebrate CNS development, early multipotent stem cells will eventually give rise to neural precursors that generate solely

neurons. Proneural bHLH factors such as Mash1 or Ngn1 are expressed in neural precursor cells in the ventricular zone and play an important role in promoting the neural fate and suppressing competence to make astroglia (Fig. 4B). For example, when Ngn1 is overexpressed in cortical progenitors in culture almost all of the cells differentiate into neurons and the astrocyte fate is suppressed (Sun *et al*., 2001). Conversely, in mice mutant for *ngn2* and *mash1*, progenitors that would normally have differentiated into neurons fail to do so and instead are biased towards differentiating as astrocytes (Nieto *et al*., 2001). Thus bHLH factors such as Ngn or Mash1 not only promote the neuronal fate but also act to suppress the astroglial fate.

The ability of proneural bHLH factors to promote neural competence was first demonstrated during nervous system development in *Drosophila*. The first step in *Drosophila* neurogenesis is to define a cluster of cells within the ectoderm with the potential to form neural precursors. This is achieved through the expression of proneural genes within a group of cells known as the proneural cluster (Cubas *et al*., 1991; Skeath and Carroll, 1991, 1992). All cells within a proneural cluster express low levels of proneural genes and have equivalent potential to become a neuroblast. Cell–cell communication through the Notch pathway (discussed in detail below) causes one cell to be selected as the neuroblast and express elevated levels of the proneural genes while the other cells adopt a non-neural epidermal fate and downregulate proneural gene expression (Fig. 3; Skeath and Carroll, 1992). If a newly delaminating neuroblast is ablated with a laser, then a neighboring cell within the equivalence group can take its place. If all cells within the equivalence group are ablated then no neuroblast forms (Taghert *et al*., 1984). Does a similar process happen in vertebrates? One important model system for understanding the function of proneural bHLH genes during vertebrate neurogenesis has been the neural plate of the amphibian embryo. Rather than being expressed in proneural clusters, early proneural bHLH genes in the *Xenopus* neural plate are expressed in broad stripes that ultimately give rise to more discrete sets of differentiated neurons within the stripes (see Fig. 4C). As discussed below, this refinement in the pattern of neurogenesis within the neural plate is mediated through the Notch signaling pathway. The first proneural bHLH gene expressed during primary neurogenesis in *Xenopus* is *X-Ngn-R1*, which is related to mammalian *ngn* (Ma *et al*., 1996). *X-Ngn-R1* in turn regulates the expression of the downstream bHLH factor NeuroD and ultimately promotes cell cycle exit and terminal neuronal differentiation. Misexpression of *X-Ngn-R1* by RNA injection into cleavage stage *Xenopus* embryos is sufficient to promote the expression of downstream genes such as *NeuroD* and convert non-neural ectodermal cells into neurons (Ma *et al*., 1996). *NeuroD* appears to be a critical regulator of the neuronal differentiation step and itself can promote the differentiation of ectopic neurons within the ectoderm when misexpressed (Fig. 4C; Lee *et al*., 1995).

Similarly, in the developing mammalian nervous system, proneural bHLH factors appear to act in a cascade that reflects the progressive stages in the neuronal differentiation process. For example, in the developing neural tube, early proneural bHLH factors such as Ngn2 are expressed in subsets of proliferating neural precursors in the ventricular zone, while later acting bHLH factors, such as Ath3/NeuroM and NeuroD are expressed in differentiating neurons as they exit the cell cycle then migrate away from the ventricular zone toward the mantle layer and become post-mitotic neurons (Lee *et al*., 1995; Roztocil *et al*., 1997). In cranial sensory neurons, Ngn1 or Ngn2 is required for the expression of NeuroM and NeuroD, which are expressed in differentiating neurons (Fode *et al*., 1998; Ma *et al*., 1998).

Proneural bHLH genes are also required for the expression of genes that are involved in the differentiation of specific neuronal subtypes. For example, in sympathetic ganglia Mash1 regulates the expression of Phox2a, which is important for acquisition of a noradrenergic phenotype (Hirsch *et al*., 1998; Lo *et al*., 1998). Thus, in addition to regulating a core program of neuronal differentiation, proneural bHLH factors may also contribute to neuronal subtype decisions. This may be modulated through cooperation with region-specific patterning factors so that the same bHLH factor can regulate the development of distinct neuronal subtypes in different regions. In the developing forebrain, for example, Mash1 regulates the development of GABAergic neurons rather than noradrenergic neurons (Letinic *et al*., 2002). As discussed below, differentiating neurons integrate multiple intrinsic and extrinsic signals to determine their ultimate phenotype.

REGULATION OF THE NUMBER OF NEURAL PROGENITORS—LATERAL INHIBITION

During vertebrate neurogenesis there is considerable spatial and temporal control over the differentiation of specific neuronal populations. Thus proneural bHLH factor activity must be constrained in some progenitors so that not all precursors differentiate simultaneously. The Notch signaling pathway plays an important role in regulating proneural bHLH factor activity and thus can control the pattern and timing of neurogenesis through a process known as lateral inhibition.

Study of invertebrates has given us much understanding of the molecular mechanisms of lateral inhibition, and these mechanisms are conserved in vertebrates. As described above, the selection of a neuroblast during *Drosophila* CNS development is governed by lateral inhibitory proteins that allow cells within an equivalence group to communicate with one another and essentially compete for the neuroblast fate. The core components of this pathway are the transmembrane Notch receptor and its transmembrane ligand Delta (Fig. 5). Activation of the Notch receptor by Delta initiates an intracellular signaling cascade that suppresses the neural fate within that cell (Artavanis-Tsakonas *et al*., 1999). This signaling pathway begins with ligand-dependent cleavage of the Notch receptor and translocation of the intracellular domain of Notch to the nucleus. There it interacts with cofactors such as Suppressor of Hairless [Su(H)] and activates transcription of bHLH repressors such as Enhancer of Split proteins [E(Spl)]. These repressors in turn inhibit expression of proneural bHLH genes and prevent cells with active Notch

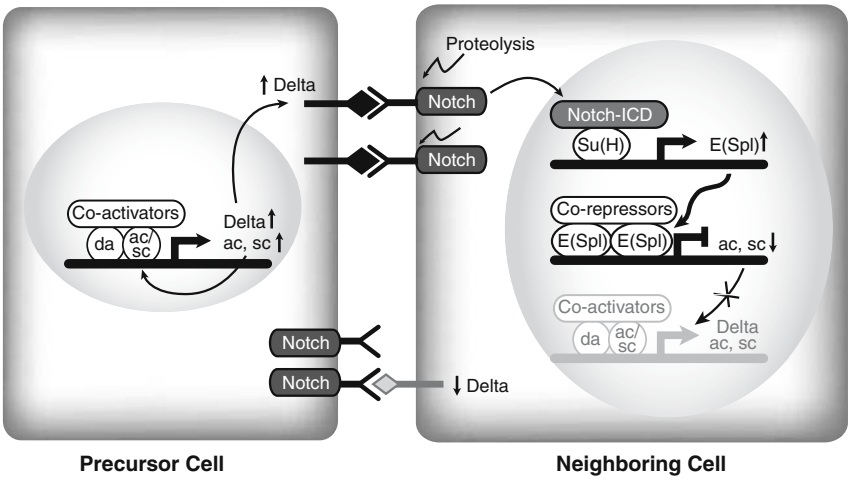

FIGURE 5. Lateral inhibition is mediated by Notch signaling between adjacent cells within a proneural cluster in the *Drosophila* neuroectoderm. Cells within the cluster express the proneural bHLH factors achaete (ac) and scute (sc), which dimerize with the bHLH partner daughterless (da), bind DNA, and regulate expression of the transmembrane ligand Delta. Delta activates the Notch receptor on adjacent cells, which initiates proteolysis of the Notch receptor and translocation of the intracellular domain (ICD) into the nucleus. Notch-ICD interacts with Suppressor of Hairless [Su(H)] and activates expression of Enhancer of Split [E(Spl)]. These repressors inhibit expression of the proneural bHLH factors causing suppression of the neuroblast fate within that cell. Loss of proneural gene expression also results in reduced Delta expression. Within a proneural cluster unknown mechanisms result in one cell (precursor cell) more strongly activating Notch signaling in neighboring cells. The neighboring cells downregulate ac/sc and Delta gene expression and ultimately differentiate into non-neural ectodermal cells. The selected precusor cell upregulates proneural gene expression through feedback autoregulation and becomes a neuroblast by Diana Lim.

signaling from adopting a neural fate. The expression of Delta in turn is positively controlled by proneural bHLH factors so that if proneural gene expression is inhibited by Notch signaling then Delta expression in that cell is also inhibited. The cell destined to become the neuroblast has slightly higher levels of Delta and thus activates Notch signaling more strongly in neighboring cells (Artavanis-Tsakonas *et al*., 1990). The selected cell has reduced Notch signaling, upregulates proneural gene expression through feedback autoregulation and in turn maintains high levels of Delta expression (Heitzler *et al*., 1996). The selected cell ultimately delaminates to become the neuroblast while the surrounding cells assume non-neural epidermal cell fates (Fig. 3). The process of lateral inhibition is fundamental to neural precursor selection throughout the developing nervous system.

Additional negative regulatory factors act outside of the proneural clusters to restrict proneural bHLH activity to only those cells within a cluster. These negative regulatory factors include bHLH factors that function as transcriptional repressors, such as Hairy (Van Doren *et al*., 1991; Ohsako *et al*., 1994), or HLH factors such as extramachrochaete (Emc) that lack a basic domain and antagonize proneural bHLH function by forming nonfunctional dimers and preventing DNA binding (Van Doren *et al*., 1991). Elimination of these negative regulators results in ectopic neuroblast formation demonstrating that these negative regulators are important for constraining proneural bHLH activity to the proneural cluster.

Identical mechanisms have been shown to operate during vertebrate neurogenesis. For example, during primary neurogenesis in *Xenopus*, the proneural bHLH factor X-Ngn-R1 promotes Delta expression, which in turn activates the Notch receptor on adjacent cells (Ma *et al*., 1996). Through the process of lateral inhibition, Notch signaling limits the number of cells that can activate expression of NeuroD and differentiate into neurons. Ectopic activation of the Notch signaling pathway inhibits primary neurogenesis, while interfering with Notch signaling results in expansion of the number of differentiating neurons within the normal domains of primary neurogenesis (Coffman *et al*., 1993; Chitnis *et al*., 1995).

Notch signaling is also important for regulating the timing of neurogenesis in the vertebrate nervous system. The components of the Notch signaling pathway in mammals are similar to *Drosophila*, with Notch receptor activation leading to upregulation of bHLH repressor genes called *Hairy/Enhancer of Split*-related genes or *Hes* genes (Davis and Turner, 2001). *Hes* genes in turn can repress the expression of proneural bHLH genes and prevent neurogenesis. *Hes1* and *Hes5* are expressed by progenitors in the VZ and mediate many effects of Notch in the developing nervous system (Kageyama and Ohtsuka, 1999). Disruption of *Hes1* causes premature neuronal differentiation (Lo *et al*., 1998), while overexpression of *Hes1* can inhibit neurogenesis (Ishibashi *et al*., 1994). Thus the *Hes* genes function as effectors of Notch activation and are important for limiting the number of neurons that differentiate at a given time.

REGULATION OF CELL NUMBER IN THE EARLY NERVOUS SYSTEM: MAINTENANCE OF A PROGENITOR POOL

Inhibition of Neuronal Differentiation

In order to generate appropriate numbers of neurons in the correct spatial and temporal patterns, it is critical to regulate progenitor cell number. This can be achieved by regulating the onset of differentiation, survival, and/or proliferation of progenitors. Stem cell and progenitor maintenance depends upon constraining the expression or function of proneural factors that act to promote neuronal differentiation. This is because proneural bHLH factors promote cell cycle exit of progenitors, which is an important step in the neuronal differentiation process. Overexpression of certain proneural bHLH factors in cell culture can promote neuronal differentiation and cell cycle exit (Farah *et al*., 2000). This may be achieved in part through upregulation of the cell cycle inhibitor $p27^{Kip1}$. Conversely, cortical progenitors isolated from *ngn2/mash1* mutant mice can proliferate much more extensively in culture than wild type progenitors, suggesting that these bHLH factors normally limit progenitor proliferation (Nieto *et al*., 2001).

Negative regulators that constrain bHLH factor expression or function are important regulators of the size of the progenitor pool since they act to prevent neuronal differentiation and cell cycle exit. In addition to coordinating the timing and pattern of neuronal differentiation, Notch signaling is also important for maintaining a population of proliferating progenitors within the VZ of the developing vertebrate neural tube. In many parts of the developing CNS, distinct neuronal subpopulations are born in the same region but at different times in development. As neurons begin to differentiate they activate Notch signaling in their neighbors, inhibit proneural gene expression or function, and thus prevent these neighboring cells from differentiating at the same time. If all progenitors were to differentiate early then the progenitor population would be depleted and later-born cell types would fail to be generated. In the developing vertebrate retina, interfering with Notch signaling by expressing a dominant negative form of the ligand Delta causes cells to preferentially adopt early-born cell fates at the expense of later-born populations (Dorsky *et al*., 1997).

A second class of negative regulators, Id proteins, can also inhibit the function of vertebrate bHLH factors and thus prevent neuronal differentiation. The *Id* genes encode HLH factors that, like Emc in *Drosophila*, lack a basic domain and antagonize proneural bHLH function by forming nonfunctional dimers with the partner E proteins, thus preventing DNA binding. *Ids* are expressed in the VZ of the developing neural tube and are important for promoting progenitor proliferation and preventing the onset of neurogenesis. For example, neural progenitors from mice mutant for both *Id1* and *Id3* show premature neuronal differentiation and cell cycle exit (Lyden *et al*., 1999). Thus Ids prevent neuronal differentiation by inhibiting proneural bHLH factor function.

Regulation of Cell Death and Proliferation

Another mechanism for regulating the size of the progenitor pool in the developing nervous system is regulation of progenitor survival. Although it has long been recognized that apoptosis is an important component of nervous system development, it was generally believed that the majority of deaths in the nervous system occurred in post-mitotic neurons as they compete for limiting amounts of trophic support from target tissue (see Chapter 11). More recently however, it has become clear that large numbers of progenitors normally die early in development, and that this is essential for regulating morphogenesis and cell number in the nervous system. This was revealed by generating mutant mice deficient for critical cell death regulators such as caspase 3, caspase 9, or Apaf1 (see Chapter 11). These mice all showed dramatic reductions in cell death in the early nervous system that resulted in severe malformations of the embryonic brain including protrusions and exencephaly of the forebrain, ventricular obstruction due to tissue hyperplasia, ectopic neural masses, and early lethality (Kuida *et al*., 1996, 1998; Yoshida *et al*., 1998). Thus, normal regulation of progenitor survival is a critical factor regulating the size of the progenitor pool during early development.

Proliferation in the early nervous system is also precisely regulated and is critical for controlling progenitor cell number. Proliferation and cell cycle exit are also intimately related to histogenesis and the neuronal cell-fate decision. Neural stem cells and progenitors respond to certain extrinsic factors with an increase in mitotic activity. For example, early neural stem cells are dependent upon FGF or EGF to proliferate and expand (Rao, 1999), while precursor cells in the cerebellum proliferate in response to Sonic hedgehog (Dahmane and Ruiz-i-Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). Proliferation in all cell types depends upon the core cell cycle machinery, including cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors, and Rb family proteins. However, it is now appreciated that these are large protein families and that different family members may play specialized roles in different tissues during development. For example, Cyclin D1 is the principal D-type cyclin regulating the transition to S-phase in the developing retina. In mice mutant for Cyclin D1, retinal progenitors show reduced proliferation (Sicinski *et al*., 1995). Conversely, CDK inhibitors such as $p27^{Kip1}$ or $p57^{Kip2}$ are expressed in retinal progenitors, and when these genes are mutated, retinal progenitors divide an extra round or two before exiting the cell cycle (Dyer and Cepko, 2000, 2001; Levine *et al*., 2000). In mice deficient for the retinoblastoma protein Rb, progenitor proliferation in the CNS is profoundly deregulated, resulting in excess dividing cells localized to normally post-mitotic regions (Dyer and Cepko, 2000, 2001; Levine *et al*., 2000). The extra cells that are generated in both these cases die by apoptosis, illustrating that cell number is regulated by the tight balance between proliferation and survival.

Asymmetric vs Symmetric Cell Division

Progenitor cell number is also dependent upon the ratio of asymmetric to symmetric cell divisions (Lu *et al*., 2000). At early

stages of development cells have been observed to undergo symmetric divisions, that is, stem cells divide perpendicular to the ventricular surface generating two daughters that both remain in contact with the ventricular surface and continue to proliferate (Chenn and McConnell, 1995). As development progresses this mode of cell division becomes less common, and the plane of cell division is more often horizontal to the ventricular surface, generating daughters that are fundamentally different from each other. One daughter remains in contact with the ventricular surface and will continue to divide. The other daughter loses contact with the ventricular surface, will exit the cell cycle, and differentiate into a post-mitotic neuron that migrates away from the VZ (Chenn and McConnell, 1995). A neural progenitor can undergo repeated asymmetric divisions generating post-mitotic daughter neurons over a prolonged developmental period. Since neural progenitors have a limited capacity for self-renewal, the progenitor will ultimately undergo a final division, which can be asymmetric, generating two nonidentical, post-mitotic daughters.

The molecular basis for asymmetric cell division was first described in *Drosophila*, where it was shown that cell-fate determinants such as Numb and Prospero function as key components in this process. During asymmetric division in *Drosophila*, Numb and Prospero proteins are localized in a crescent to one half of a dividing cell and are then asymmetrically inherited, generating two nonequivalent daughters (Jan and Jan, 2001). For example, neuroblasts in the *Drosophila* CNS undergo a series of asymmetric divisions, in each case generating another neuroblast and a GMC (see above). As the neuroblast divides, Numb and Prospero become localized to one half of the cell and are inherited by the GMC (Hirata *et al*., 1995; Knoblich *et al*., 1995; Spana and Doe, 1995). The GMC in turn can divide asymmetrically producing two post-mitotic daughters that acquire distinct neuronal or glial fates. Loss of Numb results in both daughters adopting identical fates. In *Drosophila*, Numb acts in part by antagonizing the activity of Notch, which is also required for generating two nonidentical daughters (Frise *et al*., 1996; Spana and Doe, 1996). Prospero is a homeodomain transcription factor that regulates the fate of the cell that inherits it. The localization of these determinants is regulated by a complex signaling pathway that controls the polarity of the dividing cell and the plane of cell division.

Vertebrate homologs of the Numb protein have been identified, and vertebrate Numb proteins can also be asymmetrically localized during cell division in the vertebrate CNS (Zhong *et al*., 1996). Multiple Numb family members exist and may serve diverse functions; however, there is a clear requirement for these proteins in progenitor maintenance. Mice deficient for both vertebrate *numb* and *numb-like* exhibit a premature depletion of neural progenitors and early overproduction of neurons (Petersen *et al*., 2002). These excess early-born neurons eventually die, once again demonstrating that cell number is tightly regulated. In vertebrates, the relationship between Numb and Notch remains to be fully defined.

In the preceding sections, we have shown how neuronal progenitors are specified and their numbers are regulated. Generating the correct number of progenitors is an important step in assembling the ultimate structure of the nervous system. Next, we will turn to the question of neuronal cell fate and examine how a single progenitor can give rise to multiple types of neurons.

CELL-FATE SPECIFICATION—INTRINSIC AND EXTRINSIC CUES

As a cell exits the cell cycle and becomes committed to becoming a neuron, it must also decide what type of neuron it is going to be. Although many neurons express the same genes early in their development, at some point they diverge and begin to express unique genes and proteins required for their ultimate fate. An individual neuron must express the correct neurotransmitters, receptors, and intracellular signaling molecules, and make the proper axonal and dendritic connections to other cells. All of these aspects of cellular phenotype require regulated gene expression that must be acquired over a relatively short developmental time. Previously in this chapter, we have shown that the timing of progenitor differentiation has a great influence on cell fate. A major unresolved question in the field of neurogenesis is whether the general neurogenic program and specific fate specification happen simultaneously, or as two successive steps. Evidence for both possibilities exists, and ultimately it may be more informative to explore the mechanisms by which fate specification occurs.

For many years, there have been two models for the specification of cell fate—intrinsic and extrinsic. In the intrinsic model, a cell's lineage is most important. When a progenitor cell divides, its daughters inherit "determinants" consisting of mRNA or proteins that result in a specific developmental program. These determinants could be divided asymmetrically, producing different fates from a single progenitor. Extrinsic specification instead depends on the environment, primarily through secreted or cell surface molecules. In this model, the time and place of differentiation play a greater role in cell fate than its parental lineage. Ultimately, the line between intrinsic and extrinsic specification becomes blurred, because extracellular signals can cause changes in a progenitor cell that are then passed on to its daughters. Whatever the mechanism, it is clear that all neuronal precursors begin with many possible fates and are progressively limited in potential until they differentiate.

In the following sections, we will give several examples of neuronal fate specification in different model systems, illustrating how both intrinsic and extrinsic factors contribute to cell fate. We provide examples from both vertebrates and *Drosophila*, but in each case focus on the system where the molecular factors that are required for fate specification are best understood. The examples presented here do not necessarily represent the extreme possibilities—completely intrinsic or extrinsic mechanisms. Each system seems to use a mechanism that is best suited for the final organization of its nervous system, taking into account the needs for control of precision in cell number, position, and plasticity. Importantly, all these systems use a similar hierarchy of gene expression to produce an ultimate phenotype, illustrating how a common developmental program has been adapted

throughout evolution to produce specialized components of the nervous system.

MECHANISMS FOR CNS NEURONAL FATE SPECIFICATION—EXTRINSIC AND INTRINSIC CONTROL

Vertebrate Spinal Cord

The huge number of neurons generated in vertebrate nervous systems necessitates a strong role for extracellular signals in specification of neural precursor cells. During vertebrate spinal cord development, much of the positional information that goes into the process of cell-fate specification comes from environmental signals produced by surrounding tissues. As we have mentioned previously, the neural plate already has rostrocaudal and dorsoventral polarity by the time neurogenesis begins (see Chapter 3). For example, rostrocaudal identity is encoded in the CNS by the overlapping expression of Hox proteins, as a result of early patterning molecules. In addition, the secreted molecules BMP and Hedgehog, respectively, promote dorsal and ventral identity in the developing CNS at neural plate and neural tube stages (Fig. 6). These molecules appear to act as morphogens, such that cells respond differently to increasing concentrations in their environment (Liem *et al*., 1995; Roelink *et al*., 1995). Therefore, any given cell can "sense" its DV position based on relative levels of BMP and Hedgehog signaling. Importantly, cells that occupy a particular position in the neural tube, but have not yet begun to express region-specific genes, can be respecified by exposure to ectopic environmental signals.

In response to morphogen signals, region-specific transcription factors are expressed in subsets of spinal cord progenitors. Individual homeodomain and bHLH-class transcription factors are expressed in dividing cells at different DV positions, induced by BMP and Hedgehog in a dose-dependent manner (Fig. 6). In the ventral spinal cord, these genes can be divided into two classes—those that are repressed by Hedgehog and those that are activated (Briscoe *et al*., 2000). Pairs of genes comprising a member of each class of Hh-responsive factors set up mutually exclusive domains of expression by repressing each other's expression. Once each cell in the spinal cord expresses a set of region-specific transcription factors, it then exits the cell cycle and begins to express a new set of factors that control differentiation and ultimate fate (Fig. 6). One example of this process can be seen in the expression of the Mnx class of homeodomain factors in spinal motoneurons. The two homeodomain factors HB9 and MNR2 have been shown to be necessary and sufficient for motoneuron differentiation and are themselves directly regulated by Shh and region-specific homeodomain factor expression (Tanabe *et al*., 1998; Thaler *et al*., 1999). As they begin to differentiate, neurons express a complete program of cell type-specific factors that will be discussed below.

Precision in Neuronal Fate Specification

Thus, in the spinal cord a cell's position and exposure to environmental factors leads to the expression of a cascade of transcription factors that results in ultimate fate. How universal is this mechanism to the process of neurogenesis in all animals? The large number of neurons in the vertebrate CNS allows for a high degree of plasticity. Such a system is inherently "sloppy," but is also more adaptable—if a cell is incorrectly specified, the nervous system can still function. However, we have also learned much about different mechanisms to specify neural cell fate from the study of invertebrate models. In invertebrates, precise numbers of neurons must be generated in order to ensure proper connectivity and function.

In most invertebrate nervous systems, a regular array of neurons is generated during neurogenesis, each of which can be identified by position and morphology. However, even when precise organization is required, *Drosophila* has shown us that multiple mechanisms can be used to generate defined numbers of neuronal cell fates. Following are two examples from

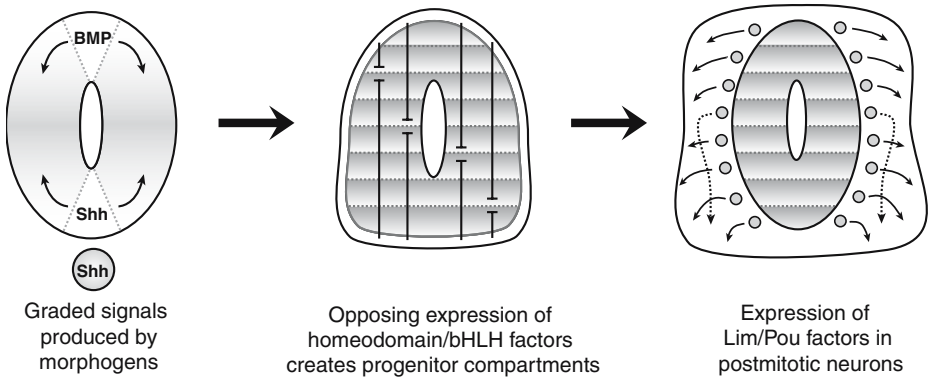

FIGURE 6. In the vertebrate spinal cord, environmental signals are translated into discrete zones of transcription factor expression that produce distinct neuronal cell types. Gradients of BMP (dorsal) and Shh (ventral) signals give each position in the spinal cord a unique dorsal/ventral identity. This identity results in the expression of particular members of homeodomain and bHLH factors, which repress each others' expression. This mutual repression creates "compartments" of progenitor cells that will produce distinct neuronal types. As neurons are born, they express type-specific transcription factors from the Lim and Pou families, which in turn regulate their differentiation. Figure generated by Diana Lim.

Drosophila, illustrating how an intrinsic timing mechanism and lineage-independent local signals can both produce predictable numbers of individual cell types.

Drosophila CNS Neuroblasts

As described previously, individual *Drosophila* neuroblasts arise from the ectoderm as a result of proneural and lateral inhibitory gene function and have a distinct positional identity based upon AP and DV patterning information (Fig. 3). Once a neuroblast identity has been specified, it divides to produce a series of GMCs and each successive GMC generates different progeny. If an individual GMC is ablated, its fate is skipped entirely and the next GMC goes on to produce daughters appropriate for its time of generation (Doe and Smouse, 1990). This therefore represents an intrinsic mechanism of fate specification. Each GMC knows its identity internally and does not depend on outside signals to learn its fate. Possible mechanisms for this type of specification include asymmetric distribution of determinants inside the cell during division, or the molecular counting of cell cycles.

There is a distinct order of transcription factors expressed in successive GMCs. In order, Hunchback, Krüppel, Pdm, and Castor are expressed first in the neuroblast, then in the subsequently generated GMC (Fig. 7). These factors appear to be necessary and sufficient in the GMCs that express them for the correct progeny to be generated (Isshiki *et al*., 2001). Interestingly, they convey a "temporal identity" on the GMC, instead of an absolute fate. As mentioned previously, neuroblasts in different positions generate different progeny, yet all their respective GMCs require these factors to produce neurons and glia appropriate for their lineage. In other words, Hunchback instructs a GMC to produce the primary fate for its position, whether that is a motoneuron or interneuron.

The *Drosophila* CNS is composed of relatively few neurons, and each makes a unique and specific connection with other neurons and muscles. Such an organization requires a high degree of precision to avoid the most serious potential problem—a missing neuron. Thus, although the initial pattern of neuroblast formation and specification is induced by environmental signals, the subsequent lineage-based system ensures that the correct number and type of each cell is produced. When a progenitor controls the fate of each of its progeny, high precision is possible.

Drosophila Retina

When many progenitor cells have the ability to produce neurons, clonally restricted lineage-dependent mechanisms are not required to generate defined numbers of mature cell types. An example of this is the *Drosophila* retina, often referred to as a "crystalline array" of ommatidia, the individual light-sensing units. Such a description is particularly illustrative of the process used to specify cell fate in this tissue. An initially uniform epithelial sheet must be patterned into a repeating array of differentiated cells, including eight photoreceptors and 12 accessory cells per ommatidium. In this case, the most important consideration is a cell's fate relative to its neighbors, rather than the presence or absence of a single cell. If one photoreceptor is missing, the fly can still see; however, if the array of ommatidia is disorganized, it cannot properly process visual information.

Differentiation proceeds across the eye imaginal disc as a wave, called the morphogenetic furrow. As this furrow moves across the disc from posterior to anterior, proneural gene activity results in a patterned array of the first photoreceptor to differentiate, R8 (Jarman *et al*., 1994). The *atonal* gene is used to specify R8 cells that are spaced apart at a proper distance through lateral inhibition by Notch/Delta signaling. These R8 cells then recruit the entire ommatidium from their neighbors, through cell–cell interactions (Fig. 8). This is a lineage independent mechanism, and it is impossible to predict which progenitor will become which photoreceptor or accessory cell before they undergo specification.

General photoreceptor specification requires a common pathway, regardless of photoreceptor cell type. Extracellular factors from the EGF family signal through tyrosine kinase receptors to the intracellular Ras-MAPK pathway, which drives the expression of transcription factors that regulate differentiation. Elimination of any part of this pathway leads to a gain of accessory cells at the expense of photoreceptors. Thus, the process of general photoreceptor differentiation, but not fate specification of R1-8, is controlled by local EGF signaling.

Once the general photoreceptor pathway is activated, local signals from differentiated cells then drive the specification of cell fate in neighboring progenitors. Photoreceptors are recruited in an invariant order—R8, then R2/5, then R3/4, then R1/6, then R7 (Fig. 8). The outer photoreceptors, R2-6, form in pairwise fashion on either side of the R8 cell. Each successive pair of photoreceptors requires specific transcription factors for its

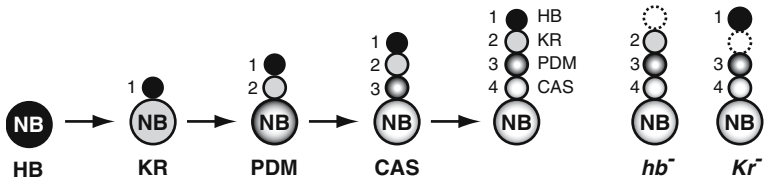

FIGURE 7. *Drosophila* CNS neurons are specified by a temporal progression of transcription factor expression. Neuroblasts express the transcription factors Hb, Kr, Pdm, and Cas at successively later timepoints during development. The neuronal progeny of these neuroblasts maintain expression of the factor that was expressed in the neuroblast when they were born. While the factors Hb and Kr are necessary and sufficient for the fates that express them, in different regions of the CNS these transcription factors drive different fates. (Modified from Isshiki *et al*., 2001, with permission from Elsevier.)

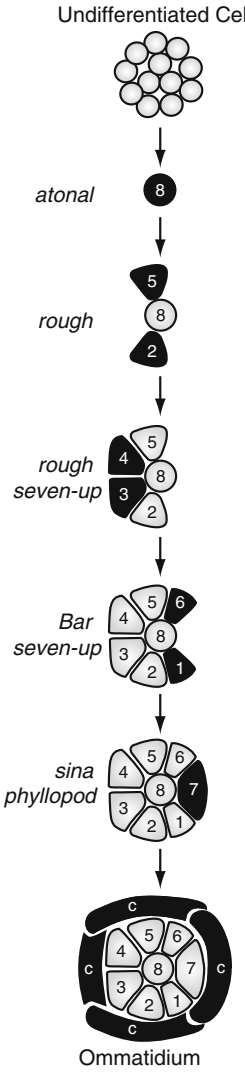

FIGURE 8. *Drosophila* ommatidial cells are recruited in a lineage-independent manner from surrounding neuroepithelium. Newly recruited cells are depicted in black. The first photoreceptor to differentiate is R8, followed in order by R2/5, R3/4, R1/6, R7, and cone cells. Genes expressed in the photoreceptors at each step are listed on the left. These genes are required for the generation of the photoreceptors in which they are expressed. Figure generated by Diana Lim.

specification. R2/5 express and require the *rough* gene, and then signal with R8 to R3/4 which requires both *rough* and *seven-up*. R1/6, the last outer photoreceptors to form, require the *seven-up* and *BarI* genes. Cell contact is required for these factors to be induced at the correct time and place, allowing one cell to control the specification of the next.

The best studied cell induction in the fly eye is formation of R7. This cell requires a combination of signals from its neighbors, which result in the expression of the correct complement of transcription factors. The EGF-Ras-MAPK pathway is activated by the ligand Boss which is expressed by R8 and activates the receptor Sevenless. The Sevenless pathway activates the ETS domain factors Pnt and AP-1 and inhibits the factor Yan, promoting general photoreceptor differentiation. Notch signaling from the neighboring R1/6 cells also plays a role in R7 specification so that Ras alone specifies the R1/6 fate, but high Ras with Notch specifies the R7 fate (Tomlinson and Struhl, 2001). Inside the R7 cell, the *lozenge* gene inhibits the expression of *seven-up*, thus preventing R1/6 differentiation. Conversely, signals from R1/6 and R8 activate genes that are required for R7 differentiation, such as *phyllopod* and *sevenless-in-absentia* (Daga *et al*., 1996). In the fly eye, cell-fate specification is therefore controlled by the time and place of differentiation. Signals from neighboring cells regulate both general and cell type-specific gene expression. Thus, local cues result in reproducible, highly organized pattern.

PLASTICITY IN FATE—VERTEBRATE CNS NEURONS

When does a neuron become irreversibly committed to a particular phenotype? One would suspect that this step takes place upon the expression of cell type-specific genes, or axon outgrowth. In fact, neurons in different organisms develop with different degrees of plasticity. In some systems, cells cannot be respecified after they leave the cell cycle. In other cases, neuronal phenotype can be respecified until a cell begins its terminal differentiation. Here we will give examples of both cases.

Cerebral Cortex—Plasticity Until Final Cell Cycle

In the mammalian cerebral cortex, control of the cell cycle appears to correspond with cells' ability to be respecified. The environment plays a key role in determining how cells know where to migrate as development progresses, and this process is dependent on the state of the cell cycle. As mentioned previously, when younger cells are transplanted into an older cortex, a subset migrates into superficial layers, appropriate for the host age (McConnell, 1988). These early cells are therefore plastic and can be influenced by their environment to adopt new fates. In the converse experiment older cells do not migrate to deeper layers when transplanted into younger animals. Thus cortical plasticity is restricted over time, with older cells becoming limited to a small number of potential fates. However, further studies have shown that the plasticity of younger progenitor cells is itself limited. While the population as a whole shows evidence of respecification in an older environment, careful analysis of single cells has uncovered diverse responses to local signals.

Labeling of cells with tritiated thymidine shows that young progenitors that have yet to go through their final S-phase adopt the fates of their older hosts and migrate into superficial layers (McConnell and Kaznowski, 1991). However, cells that have completed their final S-phase remain committed to "younger" fates and migrate to deep layers even in older hosts. Therefore, sometime after a progenitor's final S-phase, it becomes irreversibly committed to the fate promoted by its local environment.

Zebrafish Spinal Cord—Plasticity Until Axonogenesis

In the zebrafish spinal cord, cell fate commitment appears to be coupled to terminal differentiation. Environmental cues during neural tube formation initially specify these cell fates, as described in the section above. In this system, 3–4 primary motoneurons form per spinal segment, and each has a stereotypical axon trajectory and target innervation. Additionally, each primary motoneuron expresses a unique subset of LIM-homeodomain transcription factors, whose function in cell differentiation will be discussed in the following section. However, experimental manipulations have shown that motoneuron identity is not fixed until the cells begin to put out axons.

If a zebrafish primary motor neuron is transplanted to a new location before axon outgrowth, it is respecified to express LIM genes appropriate for its new position (Appel *et al*., 1995). Additionally, the axon projection of the transplanted cell follows a pathway equivalent to other neurons in the same location (Fig. 9). However, once the axon begins to grow, transplanted cells retain their original LIM gene expression and axon projection. For these cells, therefore, axonogenesis is the time when cells are irreversibly committed to a fate. From a developmental perspective, this timing makes sense because axon growth cones must express molecules on their surface to enable proper pathfinding. Once a cell switches fate, these molecules would have to be recycled and new ones expressed to allow for a new trajectory. Because all the primary motoneurons use the same neurotransmitters and function in similar circuits, gene expression before axonogenesis may be very similar between different cells and thus plasticity is possible.

Whenever extracellular signals play a role in cell-fate specification, one can measure the timing of commitment to a particular phenotype by challenging them with a new environment. By performing the above experiment *in vivo*, the researchers were able to determine the exact point at which signals in the embryo tell primary motoneurons which fate to produce. This could also be defined as the point at which extrinsic specification stops and intrinsic specification takes over, at least for some aspects of motoneuron phenotype. As we will see in a following section, other neuronal characteristics may still be plastic at this point and are regulated by target innervation. In all model systems described, this switch from extrinsic to intrinsic control happens at a slightly different point—but it happens nonetheless.

NEURONAL MATURATION

Once neurons have decided to exit the cell cycle and their fate has been specified, they undergo a process of maturation, which ultimately results in their final phenotype. As with every other event we have discussed so far, this process is controlled by gene expression. The complement of transcription factors expressed by a neural precursor cell as it differentiates will control its production of neurotransmitters and their receptors, axon guidance molecules that will regulate target innervation, and trophic dependence. The expression of these factors is a direct result of the specification process outlined in the previous section—the spatial and temporal history of each cell contributes to a "code" of transcription factors for each neuronal type that directly promotes all the above characteristics. We will give several examples of how these genes can ultimately regulate neuronal function by affecting maturation.

POU Genes Control Sensory Neurogenesis

Once they have been specified, there appears to be a conserved program of gene expression in all animal sensory neurons. Genes encoding transcription factors of the POU-homeodomain family are expressed in sensory neurons from worms to mammals. Functional analysis of these genes has demonstrated that they are necessary and sufficient to regulate sensory neurogenesis in both the CNS and PNS. In mouse, the three POU domain genes *Brn-3.0*, *Brn-3.1*, and *Brn-3.2* are expressed in and control

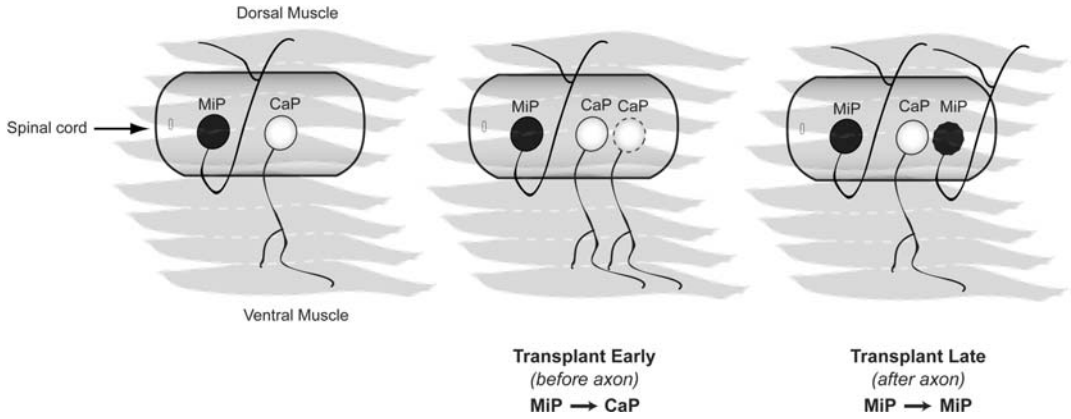

FIGURE 9. Some neurons exhibit plasticity in new environments after they are born. In the zebrafish spinal cord, the MiP primary motoneuron normally expresses Isl1 and projects dorsally, while the CaP motoneuron normally expresses Isl2 and projects ventrally. When MiP is transplanted to the CaP position before axonogenesis, it adopts a CaP phenotype. After axonogenesis, the MiP fate is fixed even when transplanted. Figure generated by Diana Lim.

the terminal differentiation of overlapping populations of sensory neuron populations. One of the clearest demonstrations of this role is in the retina, where deletion of *Brn-3.2* causes the loss of most retinal ganglion cells (Erkman *et al*., 1996). In contrast, deletion of *Brn-3.1* results in a failure of inner ear hair cells to differentiate, leading to deafness. Simultaneous deletion of *Brn-3.1* and *Brn-3.2* results in additional losses of sensory neurons, indicating that these genes play redundant roles in some populations (Wang *et al*., 2002).

What aspects of differentiation do POU-homeodomain factors regulate? Based on the phenotypes of knockouts, they act near the top of a hierarchy of gene expression that controls sensory axon formation and pathfinding. Cells in which *Brn-3* genes have been disrupted undergo cell death, rather than adopting inappropriate fates, perhaps due to a lack of trophic support from target tissues (Gan *et al*., 1999). These cells appear to begin the differentiation process before they die, indicating that they are initially specified as neurons. Retinal ganglion cells lacking *Brn-3.2* are able to migrate to the ganglion cell layer and initially extend processes that are more characteristic of dendrites than of axons. Downstream of *Brn-3.2*, target genes include members of the LIM-homeodomain family, which in turn can regulate neuronal subtype specificity, as discussed below (Erkman *et al*., 2000). These data indicate that *Brn-3.2* regulates aspects of a "projection neuron phenotype" including axon/dendrite polarity and axon guidance. While POU genes are required for terminal differentiation of sensory neurons, it is not currently clear whether there is a "code" of POU gene expression that defines each sensory subtype.

LIM Genes Regulate Subtype Specificity in CNS Motoneurons

Multiple members of the LIM homeodomain family of transcription factors are expressed in differentiating neurons of the vertebrate spinal cord. The first of these factors begins to be expressed as cells complete their final cell cycle, and others are only expressed after the final division. As mentioned in the previous section, in zebrafish primary motoneurons the expression of particular LIM factors corresponds with their axon projections. In the mouse and chick, it was also discovered that anatomically distinct pools of motoneurons express different members of this gene family, suggesting that they might contribute in some way to cellular diversity. Several of these genes are expressed in overlapping subpopulations of motoneurons: *isl1, isl2, lim1, lhx3*, and *lhx4*. One obvious way to test the role of these factors in regulating neuronal differentiation was to modulate their expression *in vivo* and examine the resulting effects on neurogenesis.

Multiple LIM genes have been knocked out in mouse, with very predictable effects on motoneuron development. *isl1* is first expressed by all motoneurons, suggesting that it activates a general program of motoneuron differentiation. When *isl1* function is removed, all motoneurons in the spinal cord are absent (Pfaff *et al*., 1996), and the precursor cells appear to undergo programmed cell death. In contrast, *lhx3* and *lhx4* are expressed transiently in a subset of motoneurons with ventral projections (Fig. 10). When these two genes are simultaneously knocked out, motoneurons still develop, but ventrally projecting neurons are lost and appear to be converted into dorsally projecting cells (Sharma *et al*., 1998). Therefore, some LIM factors may be generally required for motoneuron characteristics, while others control specific aspects of cell phenotype such as axon projection and target selection.

These same functions of LIM genes are mainly conserved in the insect nervous system as well, suggesting a common evolutionary history of neurogenesis pathways (Fig. 10). *Drosophila* CNS neurons express LIM homeodomain factors, which act to specify axon trajectories and neurotransmitter expression. In the fly, *isl*, the homologue of vertebrate *isl1* and *isl2*, is expressed by a subset of neurons in the ventral nerve cord including motoneurons. In contrast to the vertebrate spinal cord, *Drosophila* CNS neurons can still differentiate in the absence of *isl* expression, but they make errors in pathfinding and neurotransmitter expression (Thor and Thomas, 1997). This phenotype is more reminiscent of the *lhx3/4* knockout in mouse, suggesting that *isl* controls the final functional characteristics of *Drosophila* CNS neurons. In support of this role, *lim3*, the homologue of

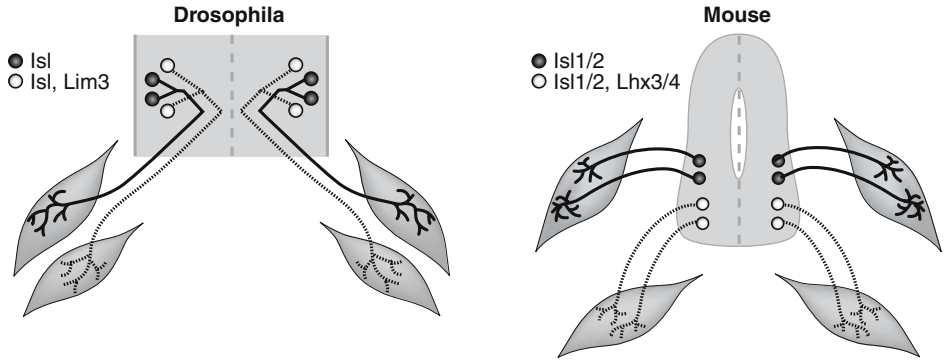

FIGURE 10. Similar LIM codes are used in fly and vertebrate motoneurons. In *Drosophila*, subsets of embryonic CNS motoneurons that express either Isl or Isl and Lim3 project to different muscles. In the vertebrate spinal cord, subsets of motoneurons express the homologues Isl1/2 or Isl1/2 and Lhx3/4 as well. As in the fly, these neurons project to different muscles depending on the combination of LIM factors they express. Figure generated by Diana Lim.

vertebrate *lhx3* and *lhx4*, is expressed in an subset of *isl*-expressing motoneurons (Fig. 10). When *lim3* expression is modified, neurons predictably adopt phenotypes characteristic of their new gene expression profile (Thor *et al*., 1999). Therefore, the combination of LIM factors expressed by a neuron gives it a unique identity that allows the proper neural connections to be made.

ETS Genes Regulate Target Specificity

The process of neuronal differentiation is not complete by the time cells send out axons and connect to their final targets. During axon pathfinding, gene expression is carefully regulated to allow growth cones to appropriately respond to local environmental cues (see Chapter 9). Even if neurons make connections, retrograde signaling from their targets can affect aspects of phenotype such as neurotransmitter expression, synaptic maturation, and cell body migration. A classic example of this regulation by targets takes place during sympathetic innervation of sweat glands, during which the neurons switch their neurotransmitter from noradrenalin to acetylcholine. It has been demonstrated that this switch is directly promoted by the target tissue, because when these neurons are forced to innervate other targets, they maintain their adrenergic phenotype. While the molecular nature of this signal has not been identified, other systems have begun to give insight into mechanisms of retrograde signaling from targets.

A molecular pathway of retrograde signaling has been observed in developing spinal motoneuron circuits. Motoneurons that innervate different targets can be subdivided into electrically coupled "pools" with common anatomical localization, gene expression, and target arborization properties. Along with common expression of the homeodomain proteins discussed previously, motoneuron pools also express the same members of the ETS family of transcription factors. One of these factors, Pea3, has been shown to be necessary for the proper axonal arborization of a subset of motoneurons in their target muscles, as well as the final migratory position of the motoneuron cell bodies (Fig. 11) (Livet *et al*., 2002). It may appear that ETS factors act in the same way as LIM factors in controlling differentiation. However, ETS expression is in fact *regulated* by motoneuron targets. When expression of the trophic factor glial-derived neurotrophic factor (GDNF) in the muscle is disrupted, motoneurons fail to express Pea3 and differentiate incorrectly (Fig. 11; Haase *et al*., 2002). Therefore, motoneurons that are genetically programmed to reach the same target subsequently receive a retrograde signal from the target that enhances their functional connectivity. Furthermore, Pea3 and a related gene ER81 have also been shown to function in proprioceptive sensory neurons that innervate muscle and connect to motoneurons in stretch reflex circuits. Because these factors regulate the connection of sensory neuron to their central targets, common expression of ETS genes in sensory and motor pools may define functional units that can be defined anatomically. What downstream targets of these factors might affect cellular connectivity? One candidate is the cadherin family of homotypic cell adhesion molecules, which are also coexpressed by common neuron pools and could regulate sorting of cell soma and axon arbors.

NEUROGENESIS IN THE ADULT VERTEBRATE NERVOUS SYSTEM

The majority of neurons in the vertebrate nervous system are generated during development through the mechanisms described above. However, there are examples of neurogenesis continuing beyond the initial embryonic period. In lower vertebrates, such as fish and amphibians, new neurons are generated during early stages of life as the animals grow. As mentioned previously, after the initial period of embryonic retinal development the retina grows in fish and amphibians by adding cells to the margins at the CMZ (Fig. 2; Straznicky and Gaze, 1971), and these new retinal cells integrate into the layers of the retina. A similar population of proliferative cells has been identified in the postnatal chick retina, but this population is lost in the adult (Fischer and Reh, 2000). In adult songbirds such as canaries, there is a seasonal replacement of neurons in specific brain nuclei involved in birdsong, in particular the HVC nucleus (Alvarez-Buylla and Kirn, 1997). New neurons are added to this

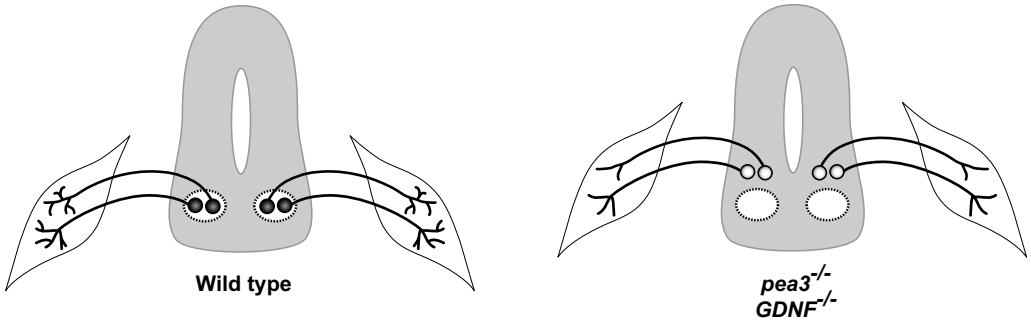

FIGURE 11. ETS factors play a role in neuronal differentiation following target innervation. Normally, a subset of vertebrate spinal motoneurons express the ETS factor Pea3 following limb muscle innervation. When Pea3 expression is lost, motoneuron cell bodies do not migrate to the correct final location, and the axons do not make appropriate synapses. The expression of Pea3 must in some part be driven by the target tissue, because loss of GDNF, expressed in the muscle, produces the same phenotype as loss of Pea3 in the neurons. Figure generated by Diana Lim.

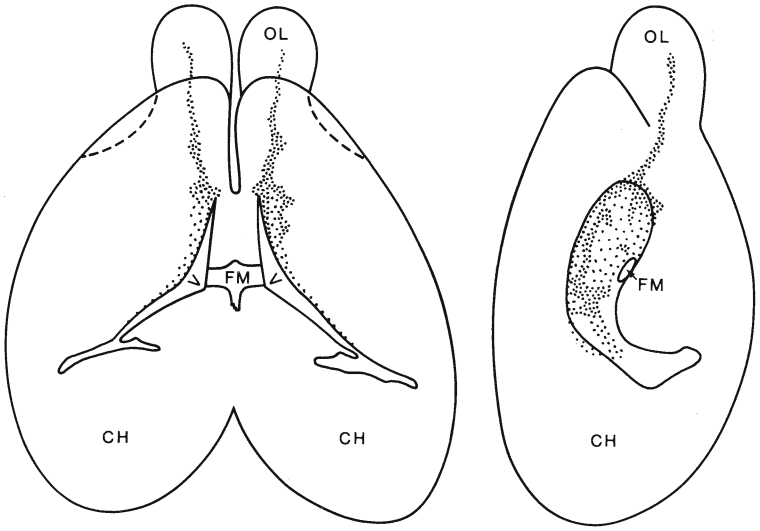

FIGURE 12. The subventricular zone (SVZ) in the telencephalon of the adult mouse. Dorsal view on the left; right lateral view on the right. The extent of the SVZ is shown by stippling and denser stippling shows where it is thickest. CH, cerebral hemisphere; FM, foramen of Monro; V, lateral ventricle; OL, olfactory lobe. From I. Smart, 1961, *J. Comp. Neurol.* 116:325–347.

nucleus in the spring, when birds learn a new song. These new neurons are born in the SVZ of the telencephalon and then migrate to populate the HVC nucleus.

Neurogenesis is much more restricted in the adult mammalian brain; however, there is now evidence that significant numbers of proliferating neural stem cells or progenitors exist within specific regions of the adult mammalian brain, and that these cells can give rise to new neurons. These regions are the subventricular zone, which lines the lateral ventricles (see Fig. 12) and the subgranular zone (SGZ) of the dentate gyrus (Taupin and Gage, 2002). SVZ progenitors give rise to interneurons that migrate along the rostral migratory stream to populate the olfactory bulb, while SGZ progenitors generate new granule cells that populate the dentate gyrus of the hippocampus. Specific stimuli can enhance neurogenesis in these brain regions. For example, exercise can stimulate the production of new neurons in the dentate gyrus (van Praag *et al*., 1999). In addition, new neurons can be generated in response to injurious events, such as ischemia or seizure, potentially contributing to a repair response in the adult brain (see Chapter 12). The molecular mechanisms underlying differentiation of adult mammalian progenitors is only partially understood, but presumably there are fundamental similarities to neurogenesis during embryonic development.

SUMMARY

In this chapter, we have outlined the general steps of neurogenesis, and the mechanisms by which these steps occur. For over a hundred years, neurobiologists have described this process by making careful observations of the cellular events that occur as neuroepithelial cells mature into neurons. These observations provided much of the groundwork for later studies and correctly predicted many of the mechanisms that were subsequently discovered. Recently, advances in molecular techniques have allowed us to understand how genetic and biochemical events control the progression from stem cells into differentiated neurons. At this point, we can observe a single neuron and know many of the genes that regulate every step of its differentiation. One important next step is to recapitulate this process *in vitro* to see if we can direct the differentiation of stem cells or progenitors in a carefully controlled environment. If successful, this work has the potential to provide therapy for human nervous system injury and disease.

REFERENCES

Akam, M., 1987, The molecular basis for metameric pattern in the *Drosophila* embryo, *Development* 101:1–22.

Alvarez-Buylla, A. and Kirn, J.R., 1997, Birth, migration, incorporation, and death of vocal control neurons in adult songbirds, *J. Neurobiol.* 33: 585–601.

Anderson, S.A., Eisenstat, D.D., Shi, L., and Rubenstein, J.L., 1997, Interneuron migration from basal forebrain to neocortex: Dependence on *dlx* genes, *Science* 278:474–476.

Angevine, J.B., 1965, Time of neuron origin in the hippocampal region: An autoradiographic study in the mouse, *Exp. Neurol. Suppl.* 2:1–70.

Angevine, J.B., Jr. and Sidman, R.L., 1961, Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse, *Nature* 192:766–768.

Appel, B., Korzh, V., Glasgow, E., Thor, S., Edlund, T., Dawid, I.B., and Eisen, J.S., 1995, Motoneuron fate specification revealed by patterned LIM homeobox gene expression in embryonic zebrafish, *Development* 121:4117–4125.

Artavanis-Tsakonas, S., Delidakis, C., Fehon, R., Hartley, D., Herndon, V., Johansen, K. *et al*., 1990, Notch and the molecular genetics of neuroblast segregation in *Drosophila*, *Mol. Reprod. Dev.* 27:23–27.

Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J., 1999, Notch signaling: Cell fate control and signal integration in development, *Science* 284: 770–776.

Austin, C.P., Feldman, D.E., Ida, J.A., Jr., and Cepko, C.L., 1995, Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch, *Development* 121:3637–3650.

Belliveau, M.J. and Cepko, C.L., 1999, Extrinsic and intrinsic factors control the genesis of amacrine and cone cells in the rat retina, *Development* 126:555–566.

Belliveau, M.J., Young, T.L., and Cepko, C.L., 2000, Late retinal progenitor cells show intrinsic limitations in the production of cell types and the kinetics of opsin synthesis, *J. Neurosci.* 20:2247–2254.

Bertrand, N., Castro, D.S., and Guillemot, F., 2002, Proneural genes and the specification of neural cell types, *Nat. Rev. Neurosci.* 3:517–530.

Bhat, K.M., 1999, Segment polarity genes in neuroblast formation and identity specification during *Drosophila* neurogenesis, *Bioessays* 21: 472–485.

Briscoe, J., Pierani, A., Jessell, T.M., and Ericson, J., 2000, A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube, *Cell* 101:435–445.

Carr, V.M. and Simpson, S.B.J., 1978, Proliferative and degenerative events in the early development of chick dorsal root ganglia. I. Normal development, *J. Comp. Neurol.* 182:727–739.

Cepko, C., 1988, Retroviruses and their applications in neurobiology, *Neuron* 1:345–353.

Cepko, C.L., Austin, C.P., Yang, X., Alexiades, M., and Ezzeddine, D., 1996, Cell fate determination in the vertebrate retina, *Proc. Natl. Acad. Sci. USA* 93:589–595.

Chenn, A. and McConnell, S.K., 1995, Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis, *Cell* 82:631–641.

Chitnis, A., Henrique, D., Lewis, J., Ish-Horowicz, D., and Kintner, C., 1995, Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene *delta*, *Nature* 375:761–766.

Coffman, C.R., Skoglund, P., Harris, W.A., and Kintner, C.R., 1993, Expression of an extracellular deletion of xotch diverts cell fate in *Xenopus* embryos, *Cell* 73:659–671.

Cubas, P., de Celis, J.F., Campuzano, S., and Modolell, J., 1991, Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc, *Genes Dev.* 5: 996–1008.

Daga, A., Karlovich, C.A., Dumstrei, K., and Banerjee, U., 1996, Patterning of cells in the *Drosophila* eye by *lozenge*, which shares homologous domains with *aml1*, *Genes Dev.* 10:1194–1205.

Dahmane, N. and Ruiz-i-Altaba, A., 1999, Sonic hedgehog regulates the growth and patterning of the cerebellum, *Development* 126: 3089–3100.

Davis, R.L. and Turner, D.L., 2001, Vertebrate Hairy and Enhancer of split related proteins: Transcriptional repressors regulating cellular dif-ferentiation and embryonic patterning, *Oncogene* 20:8342–8357.

Doe, C.Q. and Smouse, D.T., 1990, The origins of cell diversity in the insect central nervous system, *Semin. Cell Biol.* 1:211–218.

Dorsky, R.I., Chang, W.S., Rapaport, D.H., and Harris, W.A., 1997, Regulation of neuronal diversity in the *Xenopus* retina by Delta signaling, *Nature* 385:67–70.

Dyer, M.A. and Cepko, C.L., 2000, P57(kip2) regulates progenitor cell proliferation and amacrine interneuron development in the mouse retina, *Development* 127:3593–3605.

Dyer, M.A. and Cepko, C.L., 2001, P27kip1 and p57kip2 regulate proliferation in distinct retinal progenitor cell populations, *J. Neurosci.* 21: 4259–4271.

Erkman, L., McEvilly, R.J., Luo, L., Ryan, A.K., Hooshmand, F., O'Connell, S.M. *et al*., 1996, Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development, *Nature* 381:603–606.

Erkman, L., Yates, P.A., McLaughlin, T., McEvilly, R.J., Whisenhunt, T., O'Connell, S.M. *et al*., 2000, A POU domain transcription factor-dependent program regulates axon pathfinding in the vertebrate visual system, *Neuron* 28:779–792.

Farah, M.H., Olson, J.M., Sucic, H.B., Hume, R.I., Tapscott, S.J., and Turner, D.L., 2000, Generation of neurons by transient expression of neural bHLH proteins in mammalian cells, *Development* 127:693–702.

Fekete, D.M., Perez-Miguelsanz, J., Ryder, E.F., and Cepko, C.L., 1994, Clonal analysis in the chicken retina reveals tangential dispersion of clonally related cells, *Dev. Biol.* 166:666–682.

Fischer, A.J. and Reh, T.A., 2000, Identification of a proliferating marginal zone of retinal progenitors in postnatal chickens, *Dev. Biol.* 220:197–210.

Fode, C., Gradwohl, G., Morin, X., Dierich, A., LeMeur, M., Goridis, C. *et al*., 1998, The bHLH protein Neurogenin 2 is a determination factor for epibranchial placode-derived sensory neurons, *Neuron* 20:483–494.

Frantz, G.D. and McConnell, S.K., 1996, Restriction of late cerebral cortical progenitors to an upper-layer fate, *Neuron* 17:55–61.

Frise, E., Knoblich, J.A., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N., 1996, The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell–cell interaction in sensory organ lineage, *Proc. Natl. Acad. Sci. USA* 93:11925–11932.

Gan, L., Wang, S.W., Huang, Z., and Klein, W.H., 1999, Pou domain factor Brn-3b is essential for retinal ganglion cell differentiation and survival but not for initial cell fate specification, *Dev. Biol.* 210: 469–480.

Guan, W., Puthenveedu, M.A., and Condic, M.L., 2003, Sensory neuron subtypes have unique substratum preference and receptor expression before target innervation, *J. Neurosci.* 23:1781–1791.

Guillemot, F. and Joyner, A.L., 1993, Dynamic expression of the murine achaete-scute homologue *mash-1* in the developing nervous system, *Mech. Dev.* 42:171–185.

Haase, G., Dessaud, E., Garces, A., de Bovis, B., Birling, M., Filippi, P. *et al*., 2002, GDNF acts through Pea3 to regulate cell body positioning and muscle innervation of specific motor neuron pools, *Neuron* 35: 893–905.

He, W., Ingraham, C., Rising, L., Goderie, S., and Temple, S., 2001, Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis, *J. Neurosci.* 21:8854–8862.

Heitzler, P., Bourouis, M., Ruel, L., Carteret, C., and Simpson, P., 1996, Genes of the enhancer of split and achaete-scute complexes are required for a regulatory loop between Notch and Delta during lateral signalling in *Drosophila*, *Development* 122:161–171.

Henrique, D., Adam, J., Myat, A., Chitnis, A., Lewis, J., and Ish-Horowicz, D., 1995, Expression of a Delta homologue in prospective neurons in the chick, *Nature* 375:787–790.

Hirata, J., Nakagoshi, H., Nabeshima, Y., and Matsuzaki, F., 1995, Asymmetric segregation of the homeodomain protein prospero during *Drosophila* development, *Nature* 377:627–630.

Hirsch, M.R., Tiveron, M.C., Guillemot, F., Brunet, J.F., and Goridis, C., 1998, Control of noradrenergic differentiation and *phox2a* expression by Mash1 in the central and peripheral nervous system, *Development* 125:599–608.

Holt, C.E., Bertsch, T.W., Ellis, H.M., and Harris, W.A., 1988, Cellular determination in the *Xenopus* retina is independent of lineage and birth date, *Neuron* 1:15–26.

Ishibashi, M., Moriyoshi, K., Sasai, Y., Shiota, K., Nakanishi, S., and Kageyama, R., 1994, Persistent expression of helix-loop-helix factor Hes-1 prevents mammalian neural differentiation in the central nervous system, *EMBO J.* 13:1799–1805.

Isshiki, T., Pearson, B., Holbrook, S., and Doe, C.Q., 2001, *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny, *Cell* 106:511–521.

Jan, Y.N. and Jan, L.Y., 2001, Asymmetric cell division in the *Drosophila* nervous system, *Nat. Rev. Neurosci.* 2:772–779.

Jarman, A.P., Grell, E.H., Ackerman, L., Jan, L.Y., and Jan, Y.N., 1994. Atonal is the proneural gene for *Drosophila* photoreceptors, *Nature* 369:398–400.

Kageyama, R. and Ohtsuka, T., 1999, The Notch-Hes pathway in mammalian neural development, *Cell Res.* 9:179–188.

Kalyani, A., Hobson, K., and Rao, M.S., 1997, Neuroepithelial stem cells from the embryonic spinal cord: Isolation, characterization, and clonal analysis, *Dev. Biol.* 186:202–223.

Kalyani, A.J., Piper, D., Mujtaba, T., Lucero, M.T., and Rao, M.S., 1998, Spinal cord neuronal precursors generate multiple neuronal phenotypes in culture, *J. Neurosci.* 18:7856–7868.

Kalyani, A.J. and Rao, M.S., 1998, Cell lineage in the developing neural tube, *Biochem. Cell Biol.* 76:1051–1068.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N., 1995, Asymmetric segregation of Numb and Prospero during cell division, *Nature* 377:624–627.

Kuida, K., Haydar, T.F., Kuan, C.Y., Gu, Y., Taya, C., Karasuyama, H. *et al*., 1998, Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking Caspase 9, *Cell* 94:325–337.

Kuida, K., Zheng, T.S., Na, S., Kuan, C., Yang, D., Karasuyama, H. *et al*., 1996, Decreased apoptosis in the brain and premature lethality in Cpp32-deficient mice, *Nature* 384:368–372.

Lavdas, A.A., Grigoriou, M., Pachnis, V., and Parnavelas, J.G., 1999, The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex, *J. Neurosci.* 19:7881–7888.

Le Douarin, N., 1973, A biological cell labeling technique and its use in experimental embryology, *Dev. Biol.* 30:217–222.

Le Douarin, N., 1982, *The Neural Crest*, Cambridge University Press, New York.

Leber, S.M. and Sanes, J.R., 1995, Migratory paths of neurons and glia in the embryonic chick spinal cord, *J. Neurosci.* 15:1236–1248.

Lee, J.E., Hollenberg, S.M., Snider, L., Turner, D.L., Lipnick, N., and Weintraub, H., 1995, Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein, *Science* 268:836–844.

Lee, S.K. and Pfaff, S.L., 2001, Transcriptional networks regulating neuronal identity in the developing spinal cord, *Nat. Neurosci.* 4(Suppl): 1183–1191.

Letinic, K., Zoncu, R., and Rakic, P., 2002, Origin of GABAergic neurons in the human neocortex, *Nature* 417:645–649.

Levine, E.M., Close, J., Fero, M., Ostrovsky, A., and Reh, T.A., 2000, P27(kip1) regulates cell cycle withdrawal of late multipotent progenitor cells in the mammalian retina, *Dev. Biol.* 219:299–314.

Liem, K.F., Jr., Tremml, G., Roelink, H., and Jessell, T.M., 1995, Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm, *Cell* 82:969–979.

Livesey, F.J. and Cepko, C.L., 2001, Vertebrate neural cell-fate determination: Lessons from the retina, *Nat. Rev. Neurosci.* 2:109–118.

Livet, J., Sigrist, M., Stroebel, S., De Paola, V., Price, S.R., Henderson, C.E. *et al*., 2002, Ets gene *pea3* controls the central position and terminal arborization of specific motor neuron pools, *Neuron* 35:877–892.

Lo, L., Tiveron, M.C., and Anderson, D.J., 1998, Mash1 activates expression of the paired homeodomain transcription factor Phox2a, and couples pan-neuronal and subtype-specific components of autonomic neuronal identity, *Development* 125:609–620.

Lois, C. and Alvarez-Buylla, A., 1993, Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia, *Proc. Natl. Acad. Sci. USA* 90:2074–2077.

Lois, C. and Alvarez-Buylla, A., 1994, Long-distance neuronal migration in the adult mammalian brain, *Science* 264:1145–1148.

Lu, B., Jan, L., and Jan, Y.N., 2000, Control of cell divisions in the nervous system: Symmetry and asymmetry, *Annu. Rev. Neurosci.* 23:531–556.

Lyden, D., Young, A.Z., Zagzag, D., Yan, W., Gerald, W., O'Reilly, R. *et al*., 1999, Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts, *Nature* 401:670–677.

Ma, Q., Chen, Z., del Barco Barrantes, I., de la Pompa, J.L., and Anderson, D.J., 1998, Neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia, *Neuron* 20:469–482.

Ma, Q., Fode, C., Guillemot, F., and Anderson, D.J., 1999, Neurogenin1 and Neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia, *Genes Dev.* 13:1717–1728.

Ma, Q., Kintner, C., and Anderson, D.J., 1996, Identification of *neurogenin*, a vertebrate neuronal determination gene, *Cell* 87:43–52.

Mayer-Proschel, M., Kalyani, A.J., Mujtaba, T., and Rao, M.S., 1997, Isolation of lineage-restricted neuronal precursors from multipotent neuroepithelial stem cells, *Neuron* 19:773–785.

McConnell, S.K., 1988, Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation, *J. Neurosci.* 8:945–974.

McConnell, S.K. and Kaznowski, C.E., 1991, Cell cycle dependence of laminar determination in developing neocortex, *Science* 254: 282–285.

Murre, C., McCaw, P.S., and Baltimore, D., 1989a, A new DNA binding and dimerization motif in immunoglobulin enhancer binding, Daughterless, MyoD, and Myc proteins, *Cell* 56:777–783.

Murre, C., McCaw, P.S., Vaessin, H., Caudy, M., Jan, L.Y., Jan, Y.N. *et al*., 1989b, Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence, *Cell* 58:537–544.

Nieto, M., Schuurmans, C., Britz, O., and Guillemot, F., 2001, Neural bhlh genes control the neuronal versus glial fate decision in cortical progenitors, *Neuron* 29:401–413.

Nornes, H.O. and Carry, M., 1978, Neurogenesis in spinal cord of mouse: An auto radiographic analysis, *Brain Res.* 159:1–16.

O'Rourke, N.A., Chenn, A., and McConnell, S.K., 1997, Postmitotic neurons migrate tangentially in the cortical ventricular zone, *Development* 124:997–1005.

O'Rourke, N.A., Sullivan, D.P., Kaznowski, C.E., Jacobs, A.A., and McConnell, S.K., 1995, Tangential migration of neurons in the developing cerebral cortex, *Development* 121:2165–2176.

Ohsako, S., Hyer, J., Panganiban, G., Oliver, I., and Caudy, M., 1994, Hairy function as a DNA-binding helix-loop-helix repressor of *Drosophila* sensory organ formation, *Genes Dev.* 8:2743–2755.

Parnavelas, J.G., Anderson, S.A., Lavdas, A.A., Grigoriou, M., Pachnis, V., and Rubenstein, J.L., 2000, The contribution of the ganglionic eminence to the neuronal cell types of the cerebral cortex, *Novartis Found. Symp.* 228:129–139; discussion 139–147.

Perron, M., Kanekar, S., Vetter, M.L., and Harris, W.A., 1998, The genetic sequence of retinal development in the ciliary margin of the *Xenopus* eye, *Dev. Biol.* 199:185–200.

Petersen, P.H., Zou, K., Hwang, J.K., Jan, Y.N., and Zhong, W., 2002, Progenitor cell maintenance requires *numb* and *numblike* during mouse neurogenesis, *Nature* 419:929–934.

Pfaff, S.L., Mendelsohn, M., Stewart, C.L., Edlund, T., and Jessell, T.M., 1996, Requirement for LIM homeobox gene *isl1* in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation, *Cell* 84:309–320.

Qian, X., Shen, Q., Goderie, S.K., He, W., Capela, A., Davis, A.A. *et al*., 2000, Timing of CNS cell generation: A programmed sequence of neuron and glial cell production from isolated murine cortical stem cells, *Neuron* 28:69–80.

Rao, M.S., 1999, Multipotent and restricted precursors in the central nervous system, *Anat. Rec.* 257:137–148.

Rao, M.S., Noble, M., and Mayer-Proschel, M., 1998, A tripotential glial precursor cell is present in the developing spinal cord, *Proc. Natl. Acad. Sci. USA* 95:3996–4001.

Raymond, P.A. and Rivlin, P.K., 1987, Germinal cells in the goldfish retina that produce rod photoreceptors, *Dev. Biol.* 122:120–138.

Reese, B.E., Harvey, A.R., and Tan, S.S., 1995, Radial and tangential dispersion patterns in the mouse retina are cell-class specific, *Proc. Natl. Acad. Sci. USA* 92:2494–2498.

Roelink, H., Porter, J.A., Chiang, C., Tanabe, Y., Chang, D.T., Beachy, P.A. *et al*., 1995, Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of Sonic hedgehog autoproteolysis, *Cell* 81:445–455.

Roztocil, T., Matter-Sadzinski, L., Alliod, C., Ballivet, M., and Matter, J.M., 1997, NeuroM, a neural helix-loop-helix transcription factor, defines a new transition stage in neurogenesis, *Development* 124:3263–3272.

Sharma, K., Sheng, H.Z., Lettieri, K., Li, H., Karavanov, A., Potter, S. *et al*., 1998, Lim homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons, *Cell* 95:817–828.

Sicinski, P., Donaher, J.L., Parker, S.B., Li, T., Fazeli, A., Gardner, H. *et al*., 1995, Cyclin D1 provides a link between development and oncogenesis in the retina and breast, *Cell* 82:621–630.

Sidman, R.L., Miale, I.L., and Feder, N., 1959, Cell proliferation and migration in the primitive ependymal zone: An autoradiographic study of histogenesis in the nervous system, *Exp. Neurol.* 1:322–333.

Skeath, J.B. and Carroll, S.B., 1991, Regulation of achaete-scute gene expression and sensory organ pattern formation in the *Drosophila* wing, *Genes Dev.* 5:984–995.

Skeath, J.B. and Carroll, S.B., 1992, Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo, *Development* 114:939–946.

Spana, E.P. and Doe, C.Q., 1995, The Prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*, *Development* 121:3187–3195.

Spana, E.P. and Doe, C.Q., 1996, Numb antagonizes Notch signaling to specify sibling neuron cell fates, *Neuron* 17:21–26.

Straznicky, K. and Gaze, R.M., 1971, The growth of the retina in *Xenopus laevis:* An autoradiographic study, *J. Embryol. Exp. Morphol.* 26:67–79.

Sun, Y., Nadal-Vicens, M., Misono, S., Lin, M.Z., Zubiaga, A., Hua, X. *et al*., 2001, Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms, *Cell* 104:365–376.

Taghert, P.H., Doe, C.Q., and Goodman, C.S., 1984, Cell determination and regulation during development of neuroblasts and neurones in grasshopper embryo, *Nature* 307:163–165.

Tan, S.S., Kalloniatis, M., Sturm, K., Tam, P.P., Reese, B.E., and Faulkner-Jones, B., 1998, Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex, *Neuron* 21:295–304.

Tanabe, Y., William, C., and Jessell, T.M., 1998, Specification of motor neuron identity by the MNR2 homeodomain protein, *Cell* 95:67–80.

Taupin, P. and Gage, F.H., 2002, Adult neurogenesis and neural stem cells of the central nervous system in mammals, *J. Neurosci. Res.* 69:745–749.

Thaler, J., Harrison, K., Sharma, K., Lettieri, K., Kehrl, J., and Pfaff, S.L., 1999, Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor HB9, *Neuron* 23:675–687.

Thor, S., Andersson, S.G., Tomlinson, A., and Thomas, J.B., 1999, A LIM-homeodomain combinatorial code for motor-neuron pathway selection, *Nature* 397:76–80.

Thor, S. and Thomas, J.B., 1997, The *Drosophila islet* gene governs axon pathfinding and neurotransmitter identity, *Neuron* 18:397–409.

Tomlinson, A. and Struhl, G., 2001, Delta/Notch and Boss/Sevenless signals act combinatorially to specify the *Drosophila* R7 photoreceptor, *Mol. Cell* 7:487–495.

Turner, D.L. and Cepko, C.L., 1987, A common progenitor for neurons and glia persists in rat retina late in development, *Nature* 328:131–136.

Turner, D.L., Snyder, E.Y., and Cepko, C.L., 1990, Lineage-independent determination of cell type in the embryonic mouse retina, *Neuron* 4:833–845.

Van Doren, M., Ellis, H.M., and Posakony, J.W., 1991, The *Drosophila* Extramacrochaetae protein antagonizes sequence-specific DNA binding by Daughterless/Achaete-scute protein complexes, *Development* 113:245–255.

van Praag, H., Kempermann, G., and Gage, F.H., 1999, Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus, *Nat. Neurosci.* 2:266–270.

von Ohlen, T. and Doe, C.Q., 2000, Convergence of Dorsal, DPP, and EGFR signaling pathways subdivides the *Drosophila* neuroectoderm into three dorsal-ventral columns, *Dev. Biol.* 224:362–372.

Wallace, V.A., 1999, Purkinje-cell-derived sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum, *Curr. Biol.* 9:445–448.

Walsh, C. and Cepko, C.L., 1988, Clonally related cortical cells show several migration patterns, *Science* 241:1342–1345.

Walsh, C. and Cepko, C.L., 1992, Widespread dispersion of neuronal clones across functional regions of the cerebral cortex, *Science* 255:434–440.

Wang, S.W., Mu, X., Bowers, W.J., Kim, D.S., Plas, D.J., Crair, M.C. *et al*., 2002, Brn3b/Brn3c double knockout mice reveal an unsuspected role for Brn3c in retinal ganglion cell axon outgrowth, *Development* 129:467–477.

Wechsler-Reya, R.J. and Scott, M.P., 1999, Control of neuronal precursor proliferation in the cerebellum by Sonic hedgehog, *Neuron* 22:103–114.

Weisblat, D.A., Sawyer, R.T., and Stent, G.S., 1978, Cell lineage analysis by intracellular injection of a tracer enzyme, *Science* 239:1142–1145.

Yoshida, H., Kong, Y.Y., Yoshida, R., Elia, A.J., Hakem, A., Hakem, R. *et al*., 1998, Apaf1 is required for mitochondrial pathways of apoptosis and brain development, *Cell* 94:739–750.

Zhong, W., Feder, J.N., Jiang, M.M., Jan, L.Y., and Jan, Y.N., 1996, Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis, *Neuron* 17:43–53.