all of developmental biology: how to convert a single cell, the fertilized egg, into the highly differentiated cell types that characterize the mature organism. Only at later stages, as the neurons form complex circuits and experience modifies their connections, do principles of neural development diverge from those in other organs.

Early developmental principles are conserved not only among tissues but also across species and phyla. Indeed, much of what we know about the cellular and molecular bases of neural development in vertebrates comes from genetic studies of so-called simple organisms, most notably the fruit fly *Drosophila melanogaster* and the worm *Caenorhabditis elegans.* Nevertheless, because a main goal of studying neural development is to explain how the assembly of the nervous system underlies both human behavior and brain disorders, our description of the rules and principles of nervous system development focus primarily on vertebrate organisms.

## The Neural Tube Arises From the Ectoderm

The vertebrate embryo arises from the fertilized egg. Cell divisions initially form a ball of cells, called the morula, which then hollows out to form the blastula. Next, infoldings and growth generate the gastrula, a structure with polarity (dorsal-ventral and anterior-posterior) and three layers of cells—the endoderm, mesoderm, and ectoderm (Figure 45–1A).

The *endoderm* is the innermost germ layer that later gives rise to the gut, as well as to the lungs, pancreas, and liver. The *mesoderm* is the middle germ layer that gives rise to muscle, connective tissues, and much of the vascular system. The *ectoderm* is the outermost layer. Most of the ectoderm gives rise to the skin, but a narrow central strip flattens out to become the *neural plate* (Figure 45–1B). It is from the neural plate that the central and peripheral nervous systems arise.

Soon after the neural plate forms, it begins to invaginate, forming the *neural groove.* The folds then deepen and eventually separate from the rest of the ectoderm to form the *neural tube*, through a process called neurulation (Figure 45–1C,D). The caudal region of the neural tube gives rise to the spinal cord, whereas the rostral region becomes the brain. As the neural tube closes, cells at its junction with the overlying ectoderm are set aside to become the neural crest, which eventually gives rise to the autonomic and sensory nervous systems, as well as several non-neural cell types (Figure 45–1E).

## Secreted Signals Promote Neural Cell Fate

As with other organs, the emergence of the nervous system is the culmination of a complex molecular program that involves the tightly orchestrated expression of specific genes. For the nervous system, the first step is the formation of the neural plate from a restricted region of the ectoderm. This step reflects the outcome of an early choice that ectodermal cells have to make: whether to become neural or epidermal cells. This decision has been the subject of intense study for nearly 100 years.

Much of this work has focused on a search for signals that control the fate of ectodermal cells. We now know that two major classes of proteins work together to promote the differentiation of an ectodermal cell into a neural cell. The first are *inductive factors,* signaling molecules that are secreted by nearby cells. Some of these factors are freely diffusible and exert their actions at a distance, but others are tethered to the cell surface and act locally. The second are surface receptors that enable cells to respond to inductive factors. Activation of these receptors triggers the expression of genes encoding intracellular proteins—transcription factors, enzymes, and cytoskeletal proteins—that push ectodermal cells along the pathway to becoming neural cells.

The ability of a cell to respond to inductive signals, termed its *competence,* depends on the exact repertoire of receptors, transduction molecules, and transcription factors that it expresses. Thus, a cell's fate is determined not only by the signals to which it is exposed—a consequence of when and where it finds itself in the embryo—but also by the profile of genes it expresses as a consequence of its prior developmental history. We will see in subsequent chapters that the interaction of localized inductive signals and intrinsic cell responsiveness is evident at virtually every step throughout neural development.

### Development of the Neural Plate Is Induced by Signals From the Organizer Region

The discovery that specific signals are responsible for triggering the formation of the neural plate was the first major advance in understanding the mechanisms that pattern the nervous system. In 1924, Hans Spemann and Hilde Mangold made the remarkable observation that the differentiation of the neural plate from uncommitted ectoderm depends on signals secreted by a specialized group of cells they called the *organizer region.*

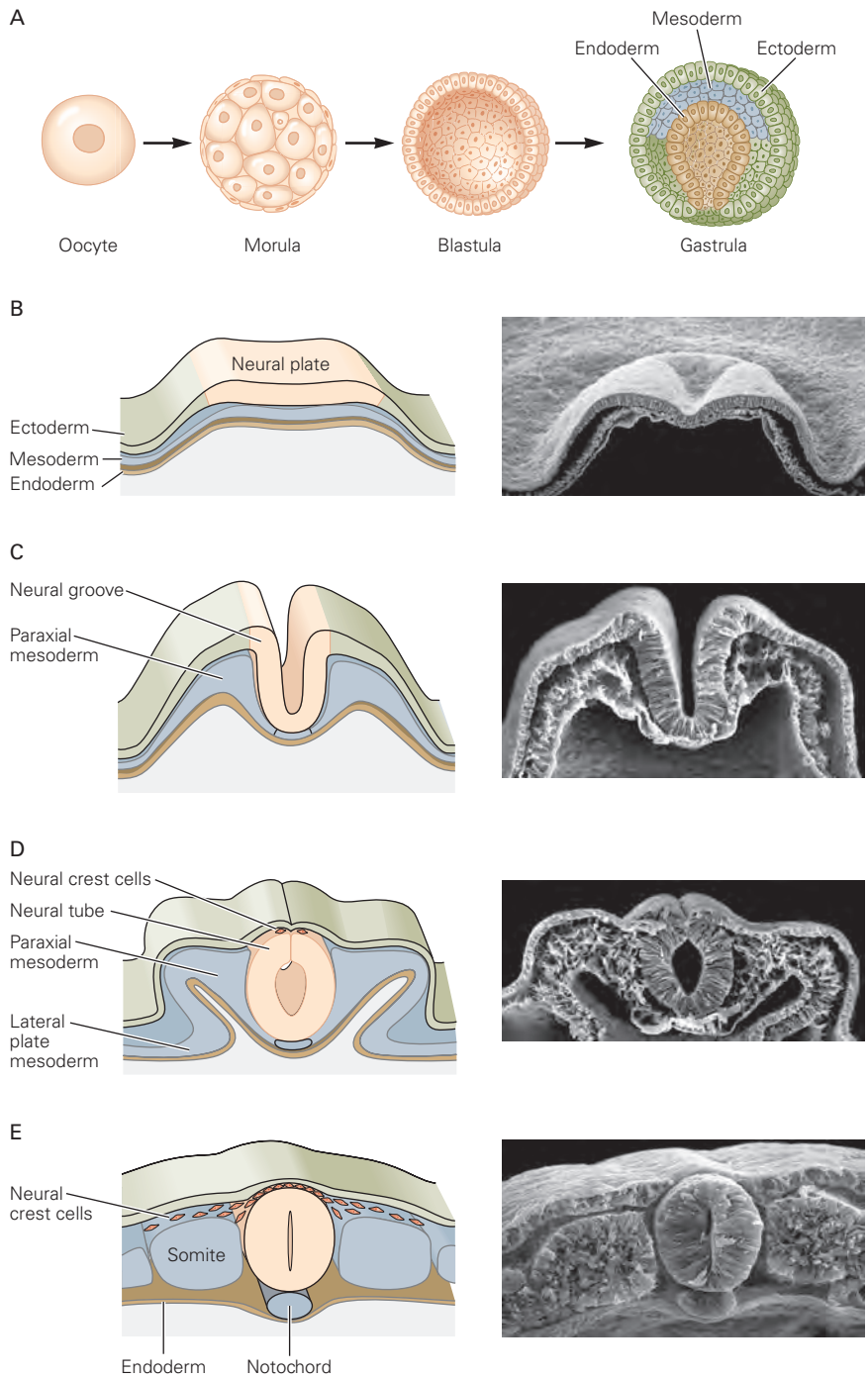


**Figure 45–1** The neural plate folds to form the neural tube. (Scanning electron micrographs of chick neural tube reproduced, with permission, from G. Schoenwolf.)

A. Following fertilization of the egg by sperm, cell divisions give rise successively to the morula, blastula, and gastrula. Three germ cell layers—the ectoderm, mesoderm, and endoderm—form during gastrulation.

B. A strip of ectoderm becomes the neural plate, the precursor of the central and peripheral nervous systems.

C. The neural plate buckles at its midline to form the neural groove.

D. Closure of the dorsal neural folds forms the neural tube.

E. The neural tube lies over the notochord and is flanked by somites, an ovoid group of mesodermal cells that give rise to muscle and cartilage. Cells at the junction between the neural tube and overlying ectoderm are set aside to become the neural crest.

Their experiments involved transplanting small pieces of tissue from one amphibian embryo to another. Most telling were transplantations of the dorsal lip of the blastopore, which is destined to form the dorsal mesoderm, from its normal dorsal position to the ventral side of a host embryo. The dorsal lip lies underneath the dorsal ectoderm, a region that normally gives rise to dorsal epidermis, including the neural plate (Figure 45–2). They grafted the tissue from a pigmented embryo into unpigmented host, allowing them to distinguish the position and fate of donor and host cells.

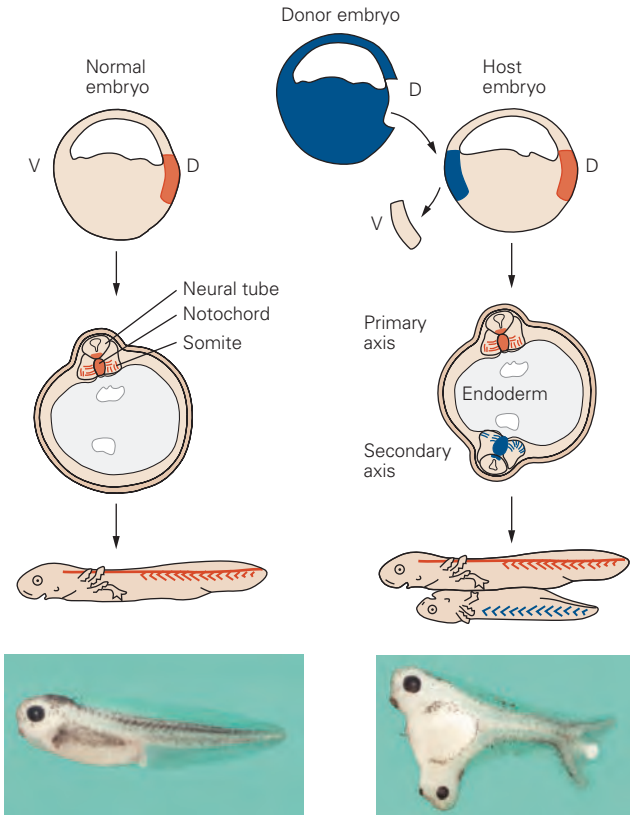


**Figure 45–2** Signals from the organizer region induce a second neural tube. (Micrographs reproduced, with permission, from Eduardo de Robertis.)

*Left:* In the normal frog, embryo cells from the organizer region (the dorsal blastopore lip) populate the notochord, floor plate, and somites. *Right:* Spemann and Mangold grafted the dorsal blastopore lip from an early gastrula stage embryo into a region of a host embryo that normally gives rise to the ventral epidermis. Signals from grafted cells induce a second embryonic axis, which includes a virtually complete neural tube. The donor tissue was from a pigmented embryo, whereas the host tissue was unpigmented, permitting the fate of grafted cells to be monitored by their color. Grafted cells themselves contribute only to the notochord, floor plate, and somites of the host embryo. As the embryo matures, the secondary neural tube develops into a complete nervous system. In the *Xenopus* embryo shown in the micrograph, the second neural axis was induced by injection of an antagonist of bone morphogenetic protein (BMP), in effect substituting for the organizer signal (Figure 45–3). The primary neural axis is also apparent. (Abbreviations: **D**, dorsal; **V**, ventral.)

Spemann and Mangold found that transplanted cells from the dorsal lip of the blastopore followed their normal developmental program, generating midline mesoderm tissue such as the somites and notochord. But the transplanted cells also caused a striking change in the fate of the neighboring ventral ectodermal cells of the host embryo. Host ectodermal cells were induced to form a virtually complete copy of the nervous system (Figure 45–2). They therefore called the donor tissue the *organizer*. Spemann and Mangold went on to show that the dorsal lip of the blastopore was the only tissue that possessed this "organizing" effect.

These pioneering studies also demonstrated that "induction" plays a critical role in neural development. Induction is a process by which cells of one tissue direct the development of neighboring cells at a region where the two come into proximity. The importance is that it provides a mechanism by which signals from one tissue can lead to subdivision of a second tissue. In this case, the mesoderm induces one part of the ectoderm to become the neural plate, and eventually the nervous system, while the remainder goes on to become epithelium, and eventually skin. The new juxtaposition thereby formed could, in principle, set the stage for a cascade of subsequent inductions and subdivisions. Indeed, we will see that many aspects of neural tube patterning are now known to depend on signals secreted by local organizing centers through actions similar in principle to that of the classical organizer region.

## Neural Induction Is Mediated by Peptide Growth Factors and Their Inhibitors

For decades after Spemann and Mangold's pioneering studies, identification of the neural inducer constituted a Holy Grail of developmental biology. The search was marked by little success until the 1980s, when the advent of molecular biology and the availability of better markers of early neural tissue led to breakthroughs in our understanding of neural induction and its chemical mediators.

The first advance came from a simple finding: When the early ectoderm is dissociated into single isolated cells, effectively preventing cell-to-cell signaling, the cells readily acquire neural properties in the absence of added factors (Figure 45–3A). The surprising implication of this finding was that the "default" fate of ectodermal cells is neural differentiation and that this fate is prevented by signaling among ectodermal cells. In this model, the long sought-after "inducer" is actually a "de-repressor": It prevents ectoderm from repressing neural fate.

These ideas immediately raised two further questions. What ectodermal signal represses neural differentiation, and what does organizer tissue provide to overcome the effects of the repressor? Studies of neural induction in frogs and chicks have now provided answers to these questions.

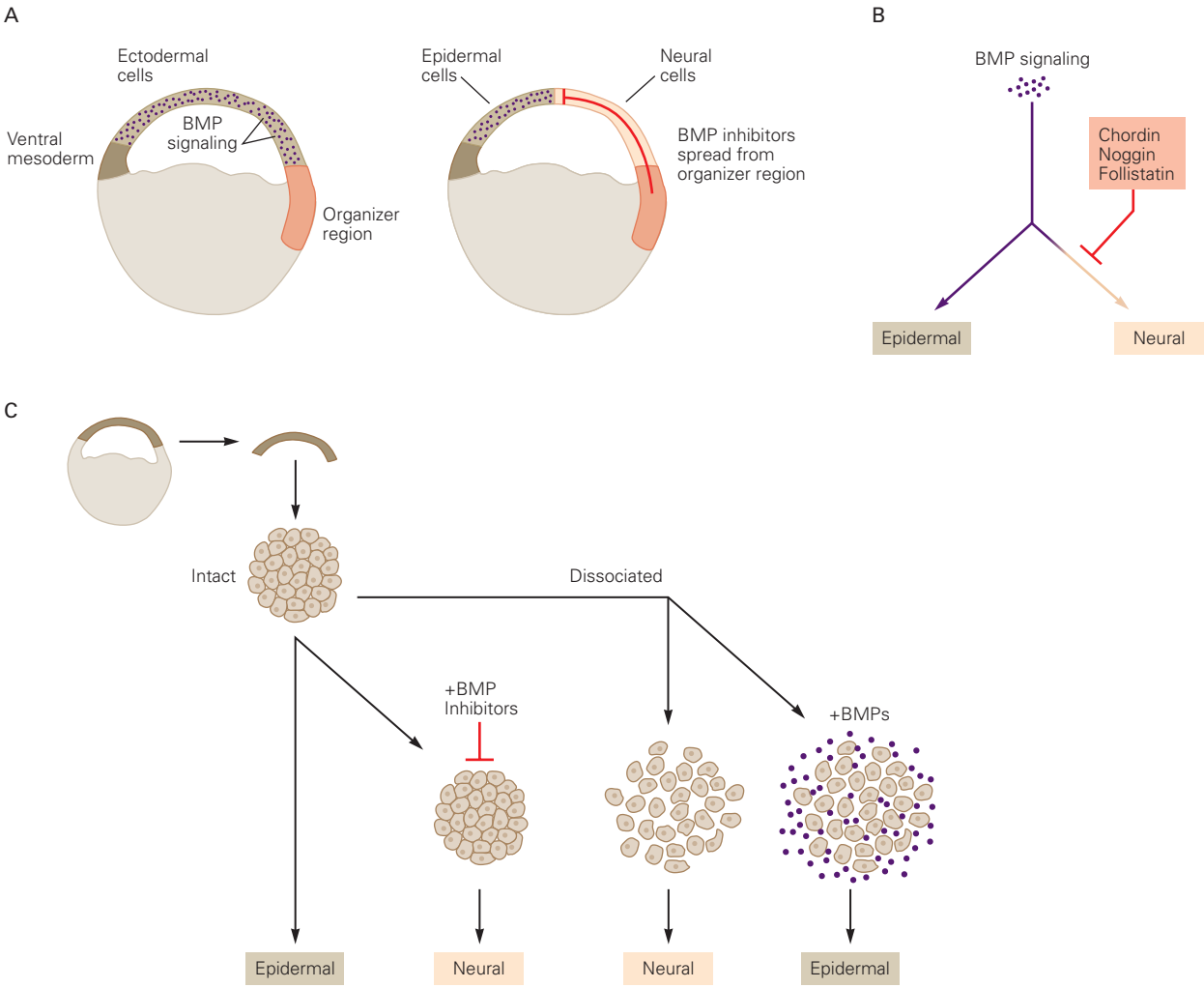


**Figure 45–3** Inhibition of bone morphogenetic protein (BMP) signaling initiates neural induction.

**A.** In *Xenopus* frog embryos, signals from the organizer region (**red line**) spread through the ectoderm to induce neural tissue. Ectodermal tissue that is beyond the range of organizer signals gives rise to epidermis.

**B.** BMP inhibitors secreted from the organizer region (including noggin, follistatin, and chordin) bind to BMPs and block the ability of ectodermal cells to acquire an epidermal fate, thus promoting neural character.

**C.** Ectodermal cells acquire neural or epidermal character depending on the presence or absence of BMP signaling. When ectodermal cell aggregates are exposed to BMP signaling, they differentiate into epidermal tissue. When BMP signaling is blocked, either by dissociating ectodermal tissue into single cells or by addition of BMP inhibitors to ectodermal cell aggregates, the cells differentiate into neural tissue.

In the absence of signals from the organizer, ectodermal cells synthesize and secrete *bone morphogenetic proteins* (BMPs), members of a large family of transforming growth factor β (TGFβ)-related proteins. The BMPs, acting through serine/threonine kinase class receptors on ectodermal cells, suppress the potential for neural differentiation and promote epidermal differentiation (Figure 43–3B). Key evidence for the role of BMPs as neural repressors came from experiments in which a truncated version of a BMP receptor, which blocks BMP signaling, was found to trigger the differentiation of neural tissue in the *Xenopus* frog embryo. Conversely, exposure of ectodermal cells to BMP signaling promoted differentiation as epidermal cells (Figure 45–3C).

The identification of BMPs as suppressors of neuronal differentiation in turn suggested that the

organizer might induce neural differentiation in ectodermal cells by secreting factors that antagonize BMP signaling. Direct support for this idea came from the finding that cells of the organizer region express many secreted proteins that act as BMP antagonists. These proteins include noggin, chordin, follistatin, and even some variant BMP proteins. Each of these proteins has the ability to induce ectodermal cells to differentiate into neural tissue (Figure 45–3B). Thus, there is no single neural inducer. In fact, multiple classes of proteins are required for induction, as shown by the later finding that the exposure of ectodermal cells to fibroblast growth factors (FGFs) is also a necessary step in neural differentiation.

Together, these studies provided a molecular explanation of the cellular phenomenon of neural induction. Although many details of the pathway remain to be clarified and some mechanistic differences among species remain perplexing, a key chapter in neural development has now been brought to a satisfying conclusion nearly a century after the organizer was discovered by Spemann and Mangold.

## Rostrocaudal Patterning of the Neural Tube Involves Signaling Gradients and Secondary Organizing Centers

As soon as cells of the neural plate have been induced, they begin to acquire regional characteristics that mark the first steps in dividing the nervous system into regions such as forebrain, midbrain, hindbrain, and spinal cord. The subdivision is directed by a series of secreted inductive factors and follows the same basic principles of neural induction. Neural plate cells in different regions of the neural tube respond to these inductive signals by expressing distinct transcription factors that gradually constrain the developmental potential of cells in each local domain. In this way, neurons in different positions acquire functional differences. Signaling occurs along both the rostrocaudal and the dorsoventral axes of the neural tube. We begin by describing rostrocaudal patterning and then return to dorsoventral patterning.

### The Neural Tube Becomes Regionalized Early in Development

After the neural tube forms, cells divide rapidly, but rates of proliferation are not uniform. Individual regions of the neural epithelium expand at different rates and begin to form the specialized parts of the mature central nervous system. Differences in the rate of proliferation of cells in rostral regions of the neural tube result in the formation of three brain vesicles: the forebrain (or prosencephalic) vesicle, the midbrain (or mesencephalic) vesicle, and the hindbrain (or rhombencephalic) vesicle (Figure 45–4A).

At this early three-vesicle stage, the neural tube flexes twice: once at the *cervical flexure,* at the junction of the spinal cord and hindbrain, and once at the *cephalic flexure,* at the junction of the hindbrain and midbrain. A third flexure, the *pontine flexure,* forms later, and later still, the cervical flexure straightens out and becomes indistinct (Figure 45–4D). The cephalic flexure remains prominent throughout development, and its persistence is the reason why the orientation of the longitudinal axis of the forebrain deviates from that of the brain stem and spinal cord.

As the neural tube develops, two of the primary embryonic vesicles divide further, thus forming five vesicles (Figure 45–4B,C). The forebrain vesicle divides to form the telencephalon, which will give rise to the cortex, hippocampus, and basal ganglia, and the diencephalon, which will give rise to the thalamus, hypothalamus, and retina. The mesencephalon, which does not divide further, gives rise to the inferior and superior colliculi and other midbrain structures. The hindbrain vesicle divides to form the metencephalon, which will give rise to the pons and cerebellum, and the myelencephalon, which will give rise to the medulla. Together with the spinal cord, these divisions make up the major functional regions of the mature central nervous system (see Chapter 4). The progressive subdivision of the neural tube into these functional domains is regulated by a variety of secreted signals.

### Signals From the Mesoderm and Endoderm Define the Rostrocaudal Pattern of the Neural Plate

It was originally believed that the organizer, as defined by Spemann and Mangold, was uniform in character, and therefore induced an initially uniform neural plate. Subsequent studies showed, however, that the organizer is regionally specialized and secretes factors that initiate the rostrocaudal patterning of the neural plate almost as soon as induction commences. One important class of factors comprises the Wnt proteins (an acronym based on their founding family members, the *Drosophila W*ingless protein and the mammalian Int1 proto-oncogene protein). Others include retinoic acid and FGFs. They are produced by mesodermal cells of the organizer as well as nearby paraxial mesoderm.

The net level of Wnt signaling activity is low at rostral levels of the neural plate and increases

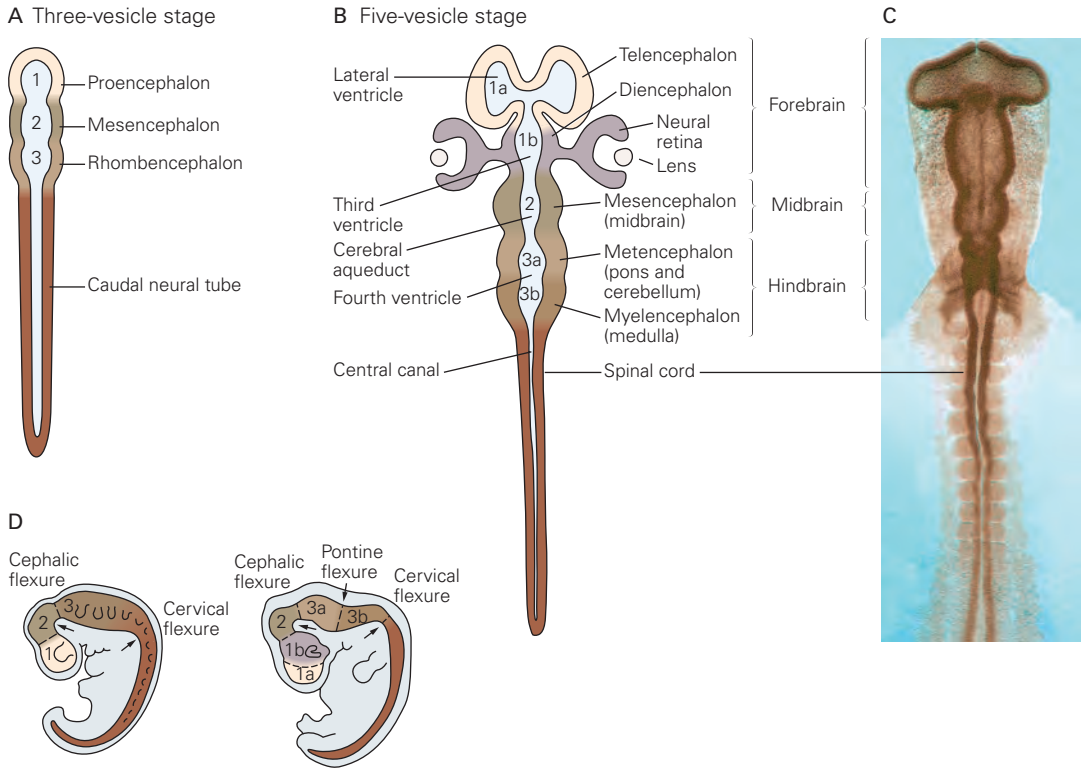


**Figure 45–4** Sequential stages of neural tube development.

A. At early stages of neural tube development, there are three brain vesicles, which will form the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain).

B. Further division within the prosencephalon and rhombencephalon generate additional vesicles. The prosencephalon splits to form the telencephalon and diencephalon, and the rhombencephalon splits to form the metencephalon and the myelencephalon.

C. Top-down view of the neural tube of a chick embryo at the five-vesicle stage. (Reproduced, with permission, from G. Schoenwolf.)

D. The neural tube bends at borders between vesicles, forming the cephalic, pontine, and cervical flexures.

progressively in the caudal direction. This activity gradient arises because the mesoderm adjacent to the caudal region of the neural plate expresses high levels of Wnt. Sharpening this gradient, tissue that underlies the rostral region of the neural plate is a source of secreted proteins that inhibit Wnt signaling, much as BMP inhibitors attenuate BMP signaling at an earlier stage. Thus, cells at progressively more caudal positions along the neural plate are exposed to increasing levels of Wnt activity and acquire a more caudal regional character, spanning the entire range from forebrain to midbrain to hindbrain and finally to spinal cord (Figure 45–5A). These results suggest that an anterior character is the "default" state for neural tissue, with signals such as Wnt imposing a posterior character. Indeed, when ectodermal cells are induced to become neural by application of BMP inhibitors, they differentiate into cells characteristic of anterior structures.

## Signals From Organizing Centers Within the Neural Tube Pattern the Forebrain, Midbrain, and Hindbrain

The early influence of mesodermal and endodermal tissues on rostrocaudal neural pattern is further refined by signals from specialized cell groups in the neural tube itself. One that has been studied in particular detail is called the *isthmic organizer*, which forms at the boundary of the hindbrain and midbrain (Figure 45–5B). The isthmic organizer serves a key role in patterning these two domains of the neural tube as well as in specifying the neuronal types within them. Dopaminergic neurons of the substantia nigra and ventral tegmental area are generated in the midbrain, just rostral to the isthmic organizer, whereas serotonergic neurons of the raphe nuclei are generated just caudal to the isthmic organizer, within the hindbrain. As an illustration of how these secondary neural signaling

centers impose neural pattern, we describe the origin and signaling activities of the isthmic organizer.

The rostrocaudal positional character of the neural plate stems from the expression of homeodomain transcription factors, the homeodomain being a section of the protein that binds to a specific DNA sequence in regulatory regions of genes, leading to changes in the gene's transcription. Cells in forebrain and midbrain domains of the neural plate express Otx2, whereas cells in the hindbrain domain express Gbx2, both of which are homeodomain transcription factors. The point of transition between Otx2 and Gbx2 expression is located at the midbrain–hindbrain boundary (Figure 45–5B) and marks the position at which the isthmic organizer will emerge after neural tube closure. At this boundary, other transcription factors are expressed, notably En1 (an Engrailed class transcription factor).

These transcription factors in turn control the expression of two signaling factors, Wnt1 and FGF8, by cells of the isthmic organizer. Wnt1 is involved in the proliferation of cells in the midbrain–hindbrain domain and in the maintenance of FGF8 expression. The spread of FGF8 from the isthmic organizer into the midbrain domain marked by Otx2 expression induces differentiation of dopaminergic neurons, whereas its spread into the hindbrain domain marked by Gbx2 expression triggers the differentiation of serotonergic neurons (Figure 45–5C).

The roles of FGF8 and Wnt1 in signaling from the *isthmic organizer* illustrate an important economy in early neural patterning. The early actions of inductive signals impose discrete domains of transcription factor expression, and these transcriptional domains then allow cells to interpret the actions of the same secreted factor in different ways, producing different neuronal subtypes. In this way, a relatively small number of secreted factors—FGFs, BMPs, hedgehog proteins, Wnt proteins, and retinoic acid—are used in different regions and at different times to program the vast diversity of neuronal cell types generated within the central and peripheral nervous systems.

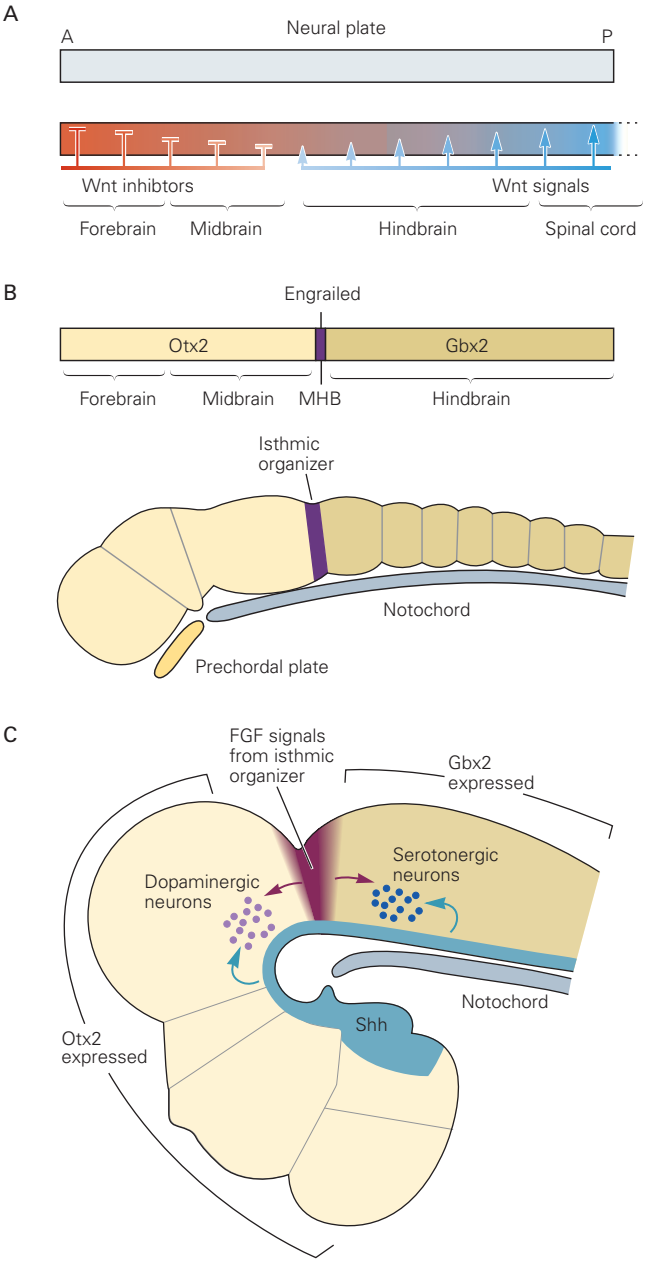


**Figure 45–5** (Left) Early anteroposterior patterning signals establish distinct transcription factor domains and define the position of the midbrain–hindbrain boundary region.

**A.** The anteroposterior pattern of the neural plate is established by exposure of neural cells to a gradient of Wnt signals. Anterior (**A**) regions of the neural plate are exposed to Wnt inhibitors secreted from the endoderm and thus perceive only low levels of Wnt activity. Progressively more posterior (**P**) regions of the neural plate are exposed to high levels of Wnt signaling from the paraxial mesoderm and to lower levels of Wnt inhibitors.

**B.** In response to this Wnt signaling gradient and other signals, cells in anterior and posterior regions of the neural plate begin to express different transcription factors: Otx2 at anterior levels and Gbx2 at more posterior levels. The intersection of these two transcription factor domains marks the region of the midbrain–hindbrain boundary (**MHB**), where Engrailed transcription factors are expressed. The neural tube then forms segments anterior and posterior to the MHB.

**C.** Fibroblast growth factor (**FGF**) signals from the isthmic organizer act in concert with sonic hedgehog (**Shh**) signals from the ventral midline to specify the identity and position of dopaminergic and serotonergic neurons. The distinct fates of these two classes of neurons result from the expression of Otx2 in the midbrain and Gbx2 in the hindbrain.

(Adapted, with permission, from Wurst and Bally-Cuif 2001. Copyright © 2001 Springer Nature.)

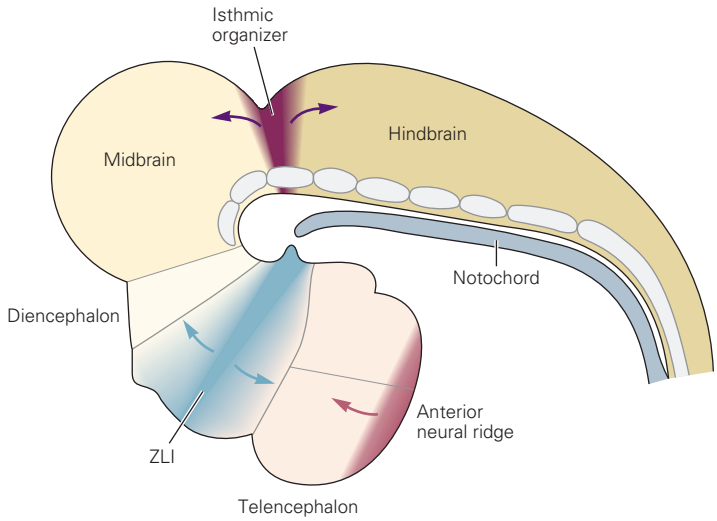


**Figure 45–6** Local signaling centers in the developing neural tube. This side view of the neural tube at a later stage shows the positions of three key signaling centers that pattern the neural tube along the anterior-posterior axis: the anterior neural ridge, the zona limitans intrathalamica (**ZLI**) at the boundary between the rostral and caudal forebrain (diencephalon), and the isthmic organizer, the boundary of the midbrain and hindbrain. The ZLI is a source of sonic hedgehog, and the isthmic organizer and anterior neural ridge are sources of fibroblast growth factor (see Figure 45–5).

Other cell groups serve similar roles in subdividing the neural tube into domains. For example, at the very rostral margin of the neural tube, a specialized group of cells, called the *anterior neural ridge*, secretes FGF that patterns the telencephalon (Figure 45–6). More caudally is a restricted region called the *zona limitans intrathalamica*, which appears as a pair of horn-like spurs within the diencephalon. Zona limitans intrathalamica cells secrete the protein sonic hedgehog (Shh), which patterns nearby cells that give rise to the nuclei of the thalamus. FGFs and Shh are described in detail below in the context of their prominent role in patterning the cortex and spinal cord, respectively.

### Repressive Interactions Divide the Hindbrain Into Segments

An important next step in patterning the neural tube along the rostrocaudal axis is the subdivision of the forebrain and hindbrain into segments, compartmental units that are arrayed along the rostrocaudal axis. These units are called *prosomeres* in the forebrain and *rhombomeres* in the hindbrain.

We use the formation of rhombomeres 3 and 4 (of 7 total) to illustrate the mechanisms leading to segmentation (Figure 45–7). An initial morphogen gradient leads to expression of two distinct transcription factors in this region—*krox20* in what will become rhombomere 3, endowing these cells with a rhombomere 3 identity, and *hoxb1* in what will become rhombomere 4, endowing these cells with a rhombomere 4 identity. Cells near the border express both factors and therefore have an uncertain identity. However, these two factors inhibit each other's expression, so eventually the identity of each cell is fixed.

The problem is that some cells are trapped within the wrong rhombomere. This intermingling is rectified in several ways, one of which is a second inhibitory interaction, this one of a markedly different type. Krox20 and Hoxb1 induce the expression of cell surface recognition and signaling molecules called EphA4 and ephrinB3, respectively. These two proteins bind to each other, leading to transmission of a repulsive signal that separates the cells. We will see below that this repulsion is also key to later decisions that axons make as they grow to their targets. In the hindbrain, before neurons form, it sharpens the borders between rhombomeres. More broadly, rhombomere segregation provides another example of a general theme in neural development: that inductive or adhesive interactions combine with repressive or inhibitory ones to pattern the nervous system.

## Dorsoventral Patterning of the Neural Tube Involves Similar Mechanisms at Different Rostrocaudal Levels

As the neural epithelium acquires its rostrocaudal character, cells located at different positions along its dorsoventral axis also begin to acquire distinct identities. Together, patterning along the rostrocaudal and dorsoventral axes divides the neural tube into a three-dimensional grid of molecularly distinct cell types, leading eventually to generation of the various neuronal and glial cell types that distinguish one part of the nervous system from another.

In contrast to the diversity of signals and organizing centers responsible for rostrocaudal patterning of developing neurons, there is a striking consistency in

the strategies and principles that establish dorsoventral pattern. We focus initially on the mechanisms of dorsoventral patterning at caudal levels of the neural tube that give rise to the spinal cord and then describe how similar strategies are used to pattern the forebrain.

Neurons in the spinal cord serve two major functions. They relay cutaneous sensory input to higher centers in the brain, and they transform sensory input into motor output. The neuronal circuits that mediate these functions are segregated anatomically. Circuits involved in the processing of cutaneous sensory information are located in the dorsal half of the spinal cord, whereas those involved in the control of motor output are mainly located in the ventral half of the spinal cord.

The neurons that form these circuits are generated at different positions along the dorsoventral axis of the spinal cord in a patterning process that begins with the establishment of distinct progenitor cell types. Motor neurons are generated close to the ventral midline, and most of the interneuron classes that control motor output are generated just dorsal to the position at which motor neurons appear (Figure 45–8). The dorsal half of the neural tube generates projection neurons and local circuit interneurons that process incoming sensory information.

How are the position and identity of spinal neurons established? The dorsoventral patterning of the neural tube is initiated by signals from mesodermal and ectodermal cells that lie close to the ventral and dorsal poles of the neural tube and is perpetuated by signals from two midline neural organizing centers. Ventral patterning signals are initially provided by the notochord, a mesodermal cell group that lies immediately under the ventral neural tube (Figure 45–1). This signaling activity is transferred to the floor plate, a specialized glial cell group that sits at the ventral midline of the neural tube itself. Similarly, dorsal signals are provided initially by cells of the epidermal ectoderm that span the dorsal midline of the neural tube, and subsequently by the roof plate, a glial cell group embedded at the dorsal midline of the neural tube (Figure 45–8).

Thus, neural patterning is initiated through a process of *homogenetic* induction, in which like begets like: Notochord signals induce the floor plate, which

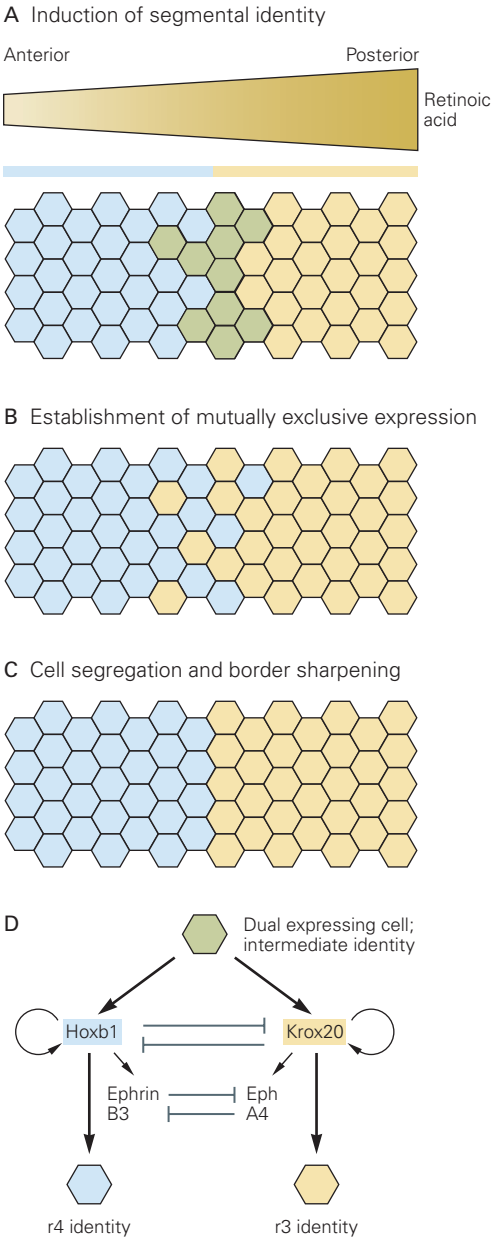


**Figure 45–7** (Left) **Repressive interactions divide the hindbrain into rhombomeres.** The sharp border between hindbrain rhombomeres 3 and 4 forms in several steps. (Adapted, with permission, from Addison and Wilkinson 2016. Copyright © 2016 Elsevier Inc.)

**A.** A gradient of retinoic acid upregulates expression of *hoxb1* in anterior cells (**blue**) and *krox20* in posterior cells (**yellow**), with some cells at the prospective border expressing both genes (**green**).

**B.** Hoxb1 expression and Krox20 expression become mutually exclusive, thus endowing each cell with a unique molecular identity.

**C.** Cells trapped in the wrong domain migrate to sharpen the border.

**D.** Inhibitory interactions underlying border formation. Hoxb1 and Krox20 repress each other's expression in individual cells, so a modest imbalance in level leads to exclusive expression of one factor. Krox20 then upregulates EphA4 in r3 cells, whereas ephrinB3 is upregulated in r4 cells. EphA4 and ephrinB3 repel each other, driving migration of isolated cells and sharpening the segment border.