CHAPTER

2

Polarity and Segmentation

REGIONAL IDENTITY OF THE NERVOUS SYSTEM

Like the rest of the body in most metazoans, the nervous system is regionally specialized. The head looks different from the tail, and the brain looks different from the spinal cord. There are a number of basic body plans for animals with neurons, and in this section, we will consider how the regional specialization of the nervous system arises during the development of some of these animals. At least some of the mechanisms that pattern the nervous system of animals are the same as those that pattern the rest of the animal's body. Similarly, many different types of tissues play key roles in regulating the development of the nervous system.

In the vertebrate embryo, most of the neural tube will give rise to the spinal cord, while the rostral end enlarges to form the three primary brain vesicles: the prosencephalon (or forebrain), the mesencephalon (or midbrain), and the rhombencephalon (or hindbrain) (Figure 2.1). The prosencephalon will give rise to the large paired cerebral hemispheres, the mesencephalon will give rise to the midbrain, and the rhombencephalon will give rise to the more caudal regions of the brainstem. The three primary brain vesicles become further subdivided into five vesicles. The prosencephalon gives rise to both the telencephalon and the diencephalon. In addition to generating the thalamus and hypothalamus in the mature brain, important features of the diencephalon are the paired evaginations of the optic vesicles. These develop into the retina and the pigmented epithelial layers of the eyes. The mesencephalon remains as a single vesicle and does not expand to the same extent as the other regions of the brain. The rhombencephalon divides into the metencephalon and the myelencephalon. These two vesicles will form the cerebellum and the medulla, respectively.

The most caudal brain region is the rhombencephalon, the region that will develop into the hindbrain. At a particular time in the development of this part of the brain, the rhombencephalon becomes divided into segments, known as rhombomeres (see below). The rhombomeres are regularly spaced repeating units of hindbrain cells and are separated by distinct boundaries. Since this is one of the clearest areas of segmentation in the vertebrate brain, study of the genes that control segmentation in rhombomeres has received a lot of attention and will be discussed in detail in the next section as a model of how the anterior-posterior patterning of the nervous system takes place in vertebrates.

The insect nervous system is made up of a series of connected ganglia known as the ventral nerve cord. In many insects, the ganglia fuse at the midline. The segmental ganglia of the ventral nerve cord are not all identical, but rather vary from anterior posterior in the number and types of neurons they contain. The insect brain is composed of three regions, known as the protocerebrum, the deutocerebrum, and the tritocerebrum (Figure 2.2). The compound eyes connect through the optic lobes to the rest of the brain. Thus, as in the vertebrate, there are quite distinct regional differences along the anterior-posterior axis of the insect nervous system, and so there must be mechanisms that make one part of the nervous system different from another part.

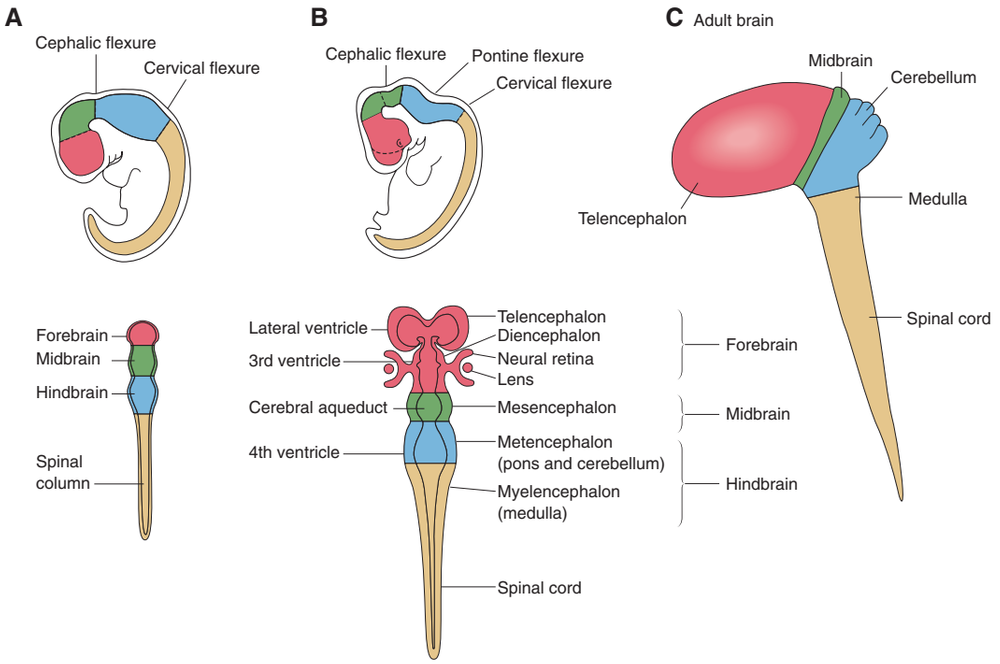

FIGURE 2.1 The vertebrate brain and spinal cord develop from the neural tube. Shown here as lateral views (upper) and dorsal views (lower) of human embryos at successively older stages of embryonic development (A,B,C). The primary three divisions of the brain (A) occur as three brain vesicles or swellings of the neural tube, known as the forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). The next stage of brain development (B) results in further subdivisions, with the forebrain vesicle becoming subdivided into the paired telencephalic vesicles and the diencephalon, and the rhombencephalon becoming subdivided into the metencephalon and the myelencephalon. These basic brain divisions can be related to the overall anatomical organization of the mature brain (C).

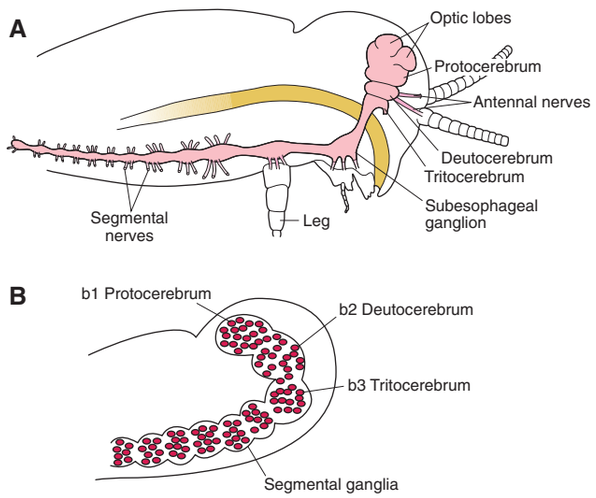

FIGURE 2.2 The brain of the *Drosophila* develops from extensive neuroblast delamination in the head. A. Three basic divisions of the brain are known as the protocerebrum, the deutocerebrum, and the tritocerebrum. B. These divisions are similar to the segmental ganglia in that they are derived independently from delaminating neuroblasts in their respective head segments. However, they later fuse together and along with the optic lobes form a complex network.

THE ANTERIOR-POSTERIOR AXIS AND *HOX* GENES

In both vertebrates and invertebrates, the mechanisms that control the regional development of the nervous system are dependent on the mechanisms that initially set up the anterior-posterior axis of the embryo. Much more is known about these mechanisms in the *Drosophila* embryo, and so this will be described first; however, it appears that many of the same genes are involved in the specification of the anterior-posterior axis in the vertebrate.

The anterior-posterior axis of the fly is primarily set up by the distribution of two molecules: a transcription factor known as *bicoid*, localized in the anterior pole of the embryo, and a gene that codes for an RNA-binding protein called *Nanos*, localized primarily in the posterior pole of the embryo (Driever and Nusslein-Volhard, 1988). The mRNAs for these genes are localized in their distribution in the egg prior to fertilization by the nurse cells in the mother. Shortly after fertilization, these mRNAs are translated, resulting in oppos-

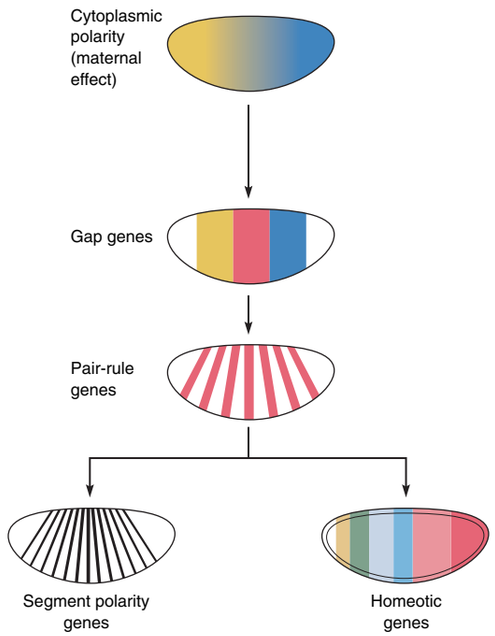

FIGURE 2.3 The unique positional identity of the segments in *Drosophila* is derived by a program of molecular steps, each of which progressively subdivides the embryo into smaller and smaller domains of expression. The oocyte has two opposing gradients of mRNA for the maternal effect genes; bicoid and hunchback are localized to the anterior half, while caudal and nanos messages are localized to the posterior regions. The maternal effect gene products regulate the expression of the gap genes, the next set of key transcriptional regulators, which are more spatially restricted in their expression. Orthodenticle (otd), for example, is a gap gene that is only expressed at the very high concentrations of bicoid present in the prospective head of the embryo. Specific combinations of the gap gene products in turn activate the transcription of the pair-rule genes, each of which is only expressed in a region of the embryo about two segments wide. The periodic pattern of the pair-rule gene expression is directly controlled by the gap genes, and along with a second set of periodically expressed genes, the segment polarity genes determine the specific expression pattern of the *homeotic* genes. In this way, each segment develops a unique identity.

ing protein gradients of the two gene products (Figure 2.3). The levels of these two proteins determine whether a second set of genes, the gap genes, are expressed in a particular region of the embryo. The gap genes, in turn, control the striped pattern of a third set of genes, the pair rule genes. Finally, the pattern of expression of the pair rule genes controls the segment-specific expression of a fourth set of genes, the segment polarity genes. This developmental hierarchy progressively divides the embryo into smaller and smaller domains with unique identities (Small and Levine, 1991; Driever and Nusslein-Volhard, 1988). This chain of transcriptional activations produces the reproducible pattern of segmentation of the animal (Figure 2.3).

At this point in the development of the fly, the anterior-posterior axis is clearly defined, and the embryo is parceled up into domains of gene expression that correspond to the different segments of the animal. The next step requires a set of genes that will uniquely specify each segment as different from one another. The genes that control the relative identity of the different parts of *Drosophila* were discovered by Edward Lewis (1978). He found mutants of the fly that had two pairs of wings instead of the usual single pair. In normal flies, wings form only on the second thoracic segment; however, in flies with a mutation in the *ultrabithorax* gene, another pair of wings forms on the third thoracic segment. These mutations transformed the third segment into another second segment. Mutations in another one of these homeotic genes—*antennapedia*—causes the transformation of a leg into an another antenna. Elimination of all of the *hox* genes in the beetle, *Tribolium*, results in an animal in which all parts of the animal look identical (Stuart et al., 1993) (Figure 2.4). Analysis of many different types of mutations in this complex have led to the conclusion that, in insects, the homeotic genes are necessary for a given part of the animal to become morphologically different from another part.

The *Homeobox* genes in *Drosophila* are arranged in a linear array on the chromosome in the order of their expression along the anterior-posterior axis of the animal (Figure 2.5). A total of eight genes are organized on the chromosome as two complexes, the Antennapaedia (ANT-C) and Bithorax (BX-C) clusters (Duboule and Morata, 1994; Gehring, 1993). The *Homeobox* genes code for proteins of the homeodomain class of transcription factors and were the original members of this very large set of related molecules. All of the *Homeobox* proteins have a sequence of approximately 60 highly conserved amino acids. Like other types of transcription factors, the *Homeobox* proteins bind to a consensus sequence of DNA in the promoters of many other genes (Gehring, 1993; Biggin and McGinnis, 1997).

How do these genes control segmental identity in *Drosophila*? A good example is the mechanism by which the *BT-X* genes control abdominal segment identity. Insects have three pairs of legs, one on each of the thoracic segments, but none on the abdominal segments. The products of the *BT-X* gene complex are responsible for suppressing the formation of legs on the abdominal segments by the repression of a key regulatory gene necessary for leg formation, the distal-less gene. Although this kind of simple regulatory interaction occurs for some aspects of segmental identity, the *Homeobox* gene products bind to a rather short core DNA sequence of just four bases, and there are likely to be many genes that contain the sequence

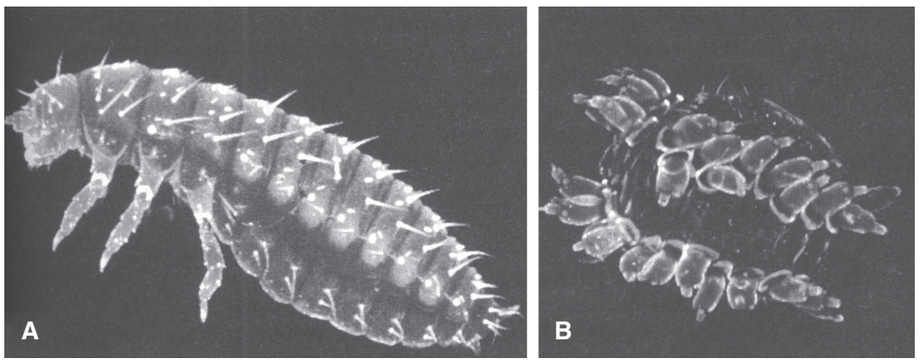

FIGURE 2.4 Elimination of the *Hox* gene cluster in the *Tribolium* beetle results in all segments developing an identical morphology. A shows the normal appearance of the beetle, and B shows an animal without a *Hox* gene cluster. The normal number of segments develop, but all of the segments acquire the morphology of the antennal segment, showing the importance of the *Hox* genes in the development of positional identity in animals. (Reproduced from Stuart et al., 1993, with permission)

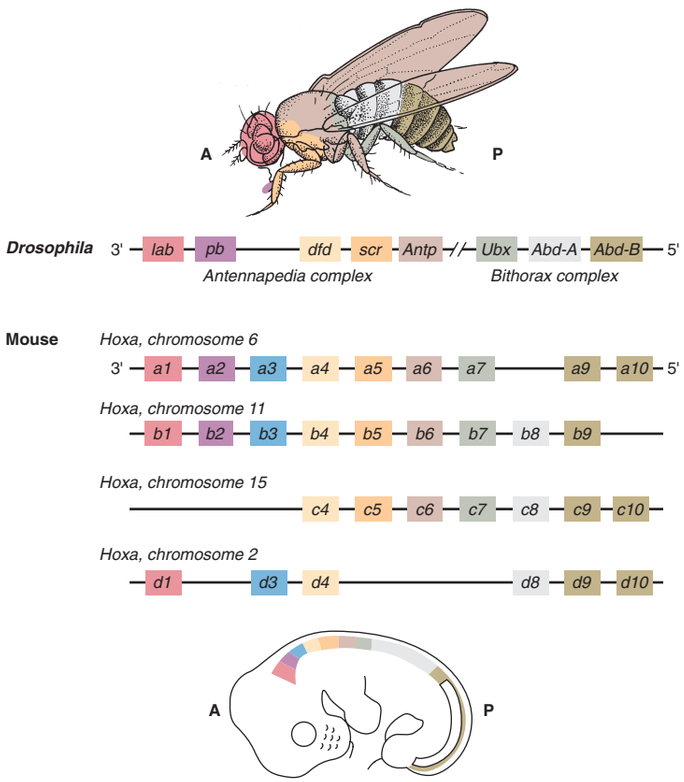

FIGURE 2.5 *Hox* gene clusters in arthropods *(Drosophila)* and vertebrates (mouse embryo) have a similar spatial organization and similar order along the chromosomes. In *Drosophila*, the *Hox* gene cluster is aligned on the chromosome such that the anterior most expressed gene is 3′ and the posterior most gene is 5′. In the mouse, there are four separate *Hox* gene clusters on four different chromosomes, but the overall order is similar to that in arthropods: the anterior to posterior order of gene expression is ordered in a 3′ to 5′ order on the chromosomes.

in their promoters, and thus are potentially regulated by *Homeobox* genes. In fact, any change in the morphology of a particular segment is likely to require the coordinated activation and suppression of numerous genes. For example, it has been estimated that between 85 and 170 genes are likely regulated by the *Ubx* gene (Gerhart and Kirschner, 1997). In addition, the *Homeobox* genes interact with other transcription factors to enhance their DNA-binding specificity.

Another striking feature of the *Homeobox* genes is their remarkable degree of conservation throughout the phyla. Organized *Homeobox* clusters similar to those found in *Drosophila* have been identified in nearly all the major classes of animals, including Cnidarians, nematodes, arthropods, annelids, and chordates. Figure 2.5 shows the relationship between the *Drosophila Homeobox* genes and those of the mouse. There have been two duplications of the ancestral *Hox* clusters to produce the A, B, C, and D clusters in the mammal. In addition, there have also been many duplications of individual members of the cluster on each chromosome, to produce up to 13 members. In mammalian embryos, the *Hox* genes are expressed in specific domains. As in *Drosophila*, the *Hox* gene position on the chromosome is correlated with its expression along the anterior-posterior axis. By aligning the mammalian *Hox* genes with their *Drosophila* counterparts, it is possible to infer the organization of the *Hox* clusters in the common ancestor between the phyla (Figure 2.5). In mice and other vertebrates, *Hox* genes in the same relative positions on each of the four chromosomes, and similar to one another in sequence, form paralogous groups. For example, *Hoxa4, Hoxb4, Hoxc4,* and *Hoxd4* make up the number 4 paralogous group.

HOX GENE FUNCTION IN THE NERVOUS SYSTEM

The function of the *Hox* genes in controlling the regional identity of the vertebrate nervous system has been most clearly investigated in the hindbrain. The vertebrate hindbrain provides the innervation for the muscles of the head through a set of cranial nerves. Like the spinal nerves that innervate the rest of the body, some of the cranial nerves contain axons from motor neurons located in the hindbrain, as well as sensory axons from neurons in the dorsal root ganglia. However, we will primarily be concerned with the motor neurons for the time being. The cranial nerves of an embryo are shown in Figure 2.6. As noted above, during embryonic development, the hindbrain undergoes a pattern of "segment formation" that bears some resemblance to that which occurs in the fly embryo. In the developing hindbrain, the segments are called rhombomeres (Figure 2.6). The rhombomeres give rise to a segmentally repeated pattern of differentiation of neurons, some of which interconnect with one another within the hindbrain (the reticular neurons) and some of which project axons into the cranial nerves (Lumsden and Keynes, 1989). Each rhombomere gives rise to a unique set of motoneurons that control different muscles in the head. For example, progenitor cells in rhombomeres 2 and 3 make the trigeminal motor neurons that innervate the jaw, while progenitor cells in rhombomeres 4 and 5 produce the motor neurons that control the muscles of facial expression (cranial nerve VII) and the neurons that control eye muscles (abducens nerve, VI), respectively. Rhombomeres 6 and 7 make the neurons of the glossopharyngeal nerve, which controls swallowing. Without differences in these segments, animals would not have differential control of smiling, chewing, swallowing or looking down. Clearly, rhombomere identity is important for our quality of life.

How do these segments become different from one another? The pattern of expression of the paralogous groups of *Hox* genes coincides with the rhombomere boundaries (Figure 2.6), and in fact the expression of these genes precedes the formation of obvious morphological rhombomeric boundaries. Members of paralogous groups 1–4 are expressed in the rhombomeres in a nested, partly overlapping pattern. Group 4 genes are expressed up to the anterior boundary of the seventh rhombomere, group 3 genes are expressed up to and including rhombomere 5, while group 2 genes are expressed in rhombomeres 2–5. These patterns are comparable in all vertebrates. As discussed below, loss of a single *Hox* gene in mice usually does not produce the sort of dramatic phenotypes seen in *Drosophila*. This is probably because of overlapping patterns of *Hox* gene expression from the members of the four paralogous groups. When two or more members of a paralogous group are deleted, say *Hoxa4* and *Hoxb4*, then the severity of the deficits increases. The deficits that are observed are consistent with the *Hox* genes acting much as they do in arthropods. That is, they control the relative identity of a region of the body.

As noted above, studies of *Hox* genes in neural development have concentrated on the hindbrain. Several studies have either deleted specific *Hox* genes or misexpressed them in other regions of the CNS and examined the effect on hindbrain development. Only a few examples will be given. Elimination of the *Hoxa1* gene from mice results in animals with defects in the

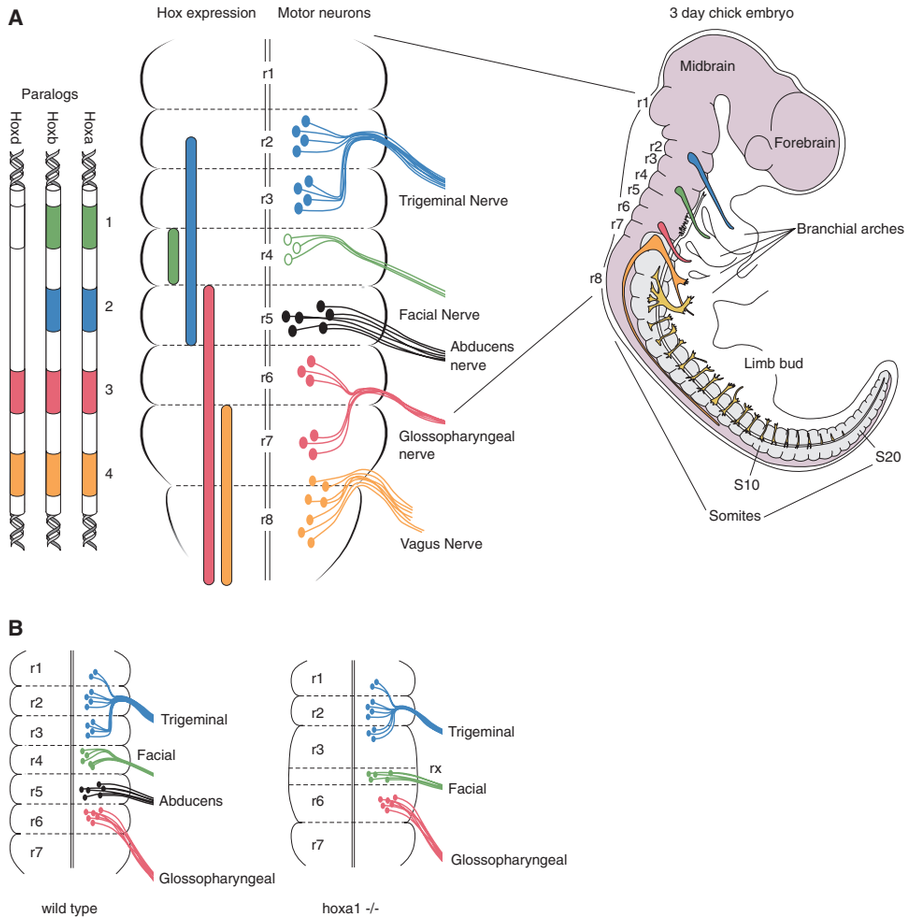

FIGURE 2.6 Rhombomeres are repeated morphological subdivisions of the hindbrain. A. The rhombomeres are numbered from the anterior-most unit, *r1*, just posterior to the midbrain (mesencephalon), to the posterior most unit, *r7*, at the junction of the hindbrain with the spinal cord. The members of the *Hox* gene cluster are expressed in a 3′ to 5′ order in the rhombomeres. The segmentation in this region of the embryo is also observed in the cranial nerves, and the motoneurons send their axons through defined points at alternating rhombomeres. B. Rhombomere identity is determined by the *Hox* code. *Hox* gene knockouts in mice affect the development of specific rhombomeres. Wild-type animals have a stereotypic pattern of motoneurons in the hindbrain. The trigeminal (V) cranial nerve motoneurons are generated from *r2* and *r3*, while the facial nerve motoneurons are produced in *r4* and the abducens motorneurons are produced by *r5*. Deletion of the *Hoxa1* gene in mice causes the complete loss of rhombomere 5 and a reduction of rhombomere 4 (rx). The abducens motoneurons are lost in the knockout animals, and the number of the facial motoneurons is reduced.

development of rhombomeres and the neurons they produce (Lufkin et al., 1993; Gavalas et al., 2003). Specifically, the rhombomere 4 domain is dramatically reduced and does not form a clear boundary with rhombomere 3. Rhombomere 5 is completely lost, or fused with rhombomere 4, into a new region called "rx." The abducens motoneurons fail to develop in these animals, and the facial motor neurons are also defective. However, some of the neurons derived from this region of the hindbrain now begin to resemble the trigeminal motor neurons (Figure 2.6). Thus, when *Hoxa1* is lost from the hindbrain, rhombomere 4 and 5 are partly transformed to a rhombomere 2/3 identity. Thus, at least at some level, the *Hox* genes of mice appear to confer regional anterior-posterior identity on a region of the nervous system in a manner similar to the homeotic genes of *Drosophila*.

Earlier in this section, we showed a picture of an arthropod that had no *Hox* genes; all segments were essentially identical. Is this true of vertebrates? What would the hindbrain look like without *Hox* genes? Studies in both *Drosophila* and vertebrates have found that the specificity of the *Hox* genes for promoters on their downstream targets is significantly enhanced

through their interactions with the *Pbx* and *Meis* homeodomain proteins. Moens and her colleagues (Waskiewisz et al., 2002) have taken advantage of this interaction to ask what the hindbrain would look like without any functional *Hox* code. By eliminating the *Pbx* genes from the hindbrain of the zebrafish with a combination of genetic mutation and antisense oligonucleotide gene inactivation, they have found that the "ground state" or default condition of the hindbrain is rhombomere number 1. Embryos lacking both *Pbx* genes that are normally expressed in the hindbrain during rhombomere formation lose rhombomeres 2–6, and instead these segments are transformed into one long rhombomere 1 (Figure 2.7).

The remarkable conservation of *Hox* gene functioning in defining segmental identity in both *Drosophila* embryos and vertebrate hindbrain prompts the question whether similar mechanisms upstream of *Hox* gene expression are also conserved. As discussed above, a developmental cascade of genes—the gap genes, the pair-rule genes, and the segment polarity genes—parcel up the domains of the fly embryo into smaller and smaller regions, each of which has a unique *Homeobox* expression pattern. Does a similar mechanism act in the vertebrate brain to control the expression of the *Hox* genes? Although the final answers are not yet known, there are several key observations that indicate vertebrates may use somewhat different mechanisms to define the pattern of *Hox* expression.

One of the first signaling molecules to be implicated as a regulator of *Hox* expression was a derivative of vitamin A, retinoic acid (RA). This molecule is a powerful teratogen; that is, it causes birth defects. Retinoic acid is a common treatment for acne, and since its introduction in 1982, approximately one thousand malformed children have been born. The most significant defects involve craniofacial and brain abnormalities. The way in which RA works is as follows: RA crosses the cell membrane to bind a cytoplasmic receptor (Figure 2.8). The complex of RA and the retinoic acid receptor (RAR) moves into the nucleus, where it can regulate gene expression through interaction with a specific sequence in the promoters of target genes (the retinoic acid response element, or RARE). In the normal embryo, there is a gradient of

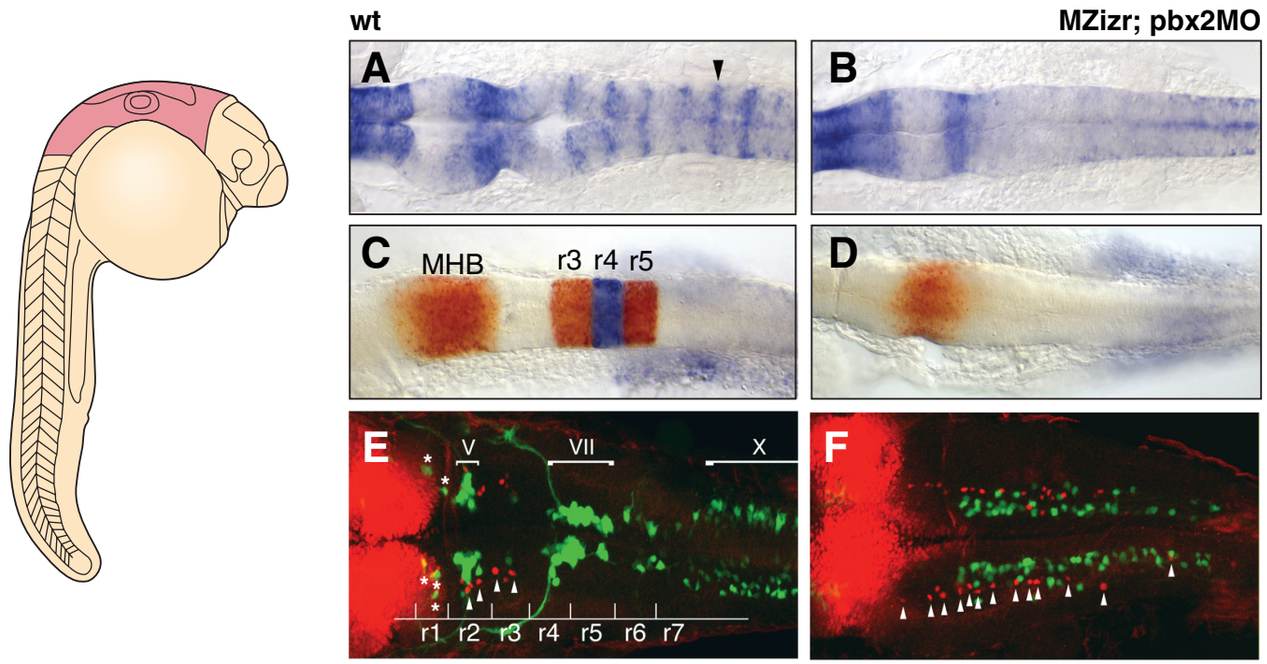

FIGURE 2.7 What would the hindbrain look like without *Hox* genes? By eliminating the *pbx* genes from the hindbrain of the zebrafish with a combination of genetic mutation and antisense oligonucleotide gene inactivation, Moens and colleagues found that the "ground state" or default condition of the hindbrain is rhombomere number 1. To the right is a drawing of the fish for orientation, with the hindbrain highlighted in red. A, C, and E show the wild-type embryo, and panels B, D, F show the mutant embryo hindbrain. In embryos lacking both *pbx* genes all segments are transformed into one long rhombomere 1, and both the specific gene expression seen in rhombomeres 3, 4, and 5 (D) and the diversity of neurons that form in the hindbrain (E) are lost in the mutant. (Modified from Waskiewicz et al., 2002)

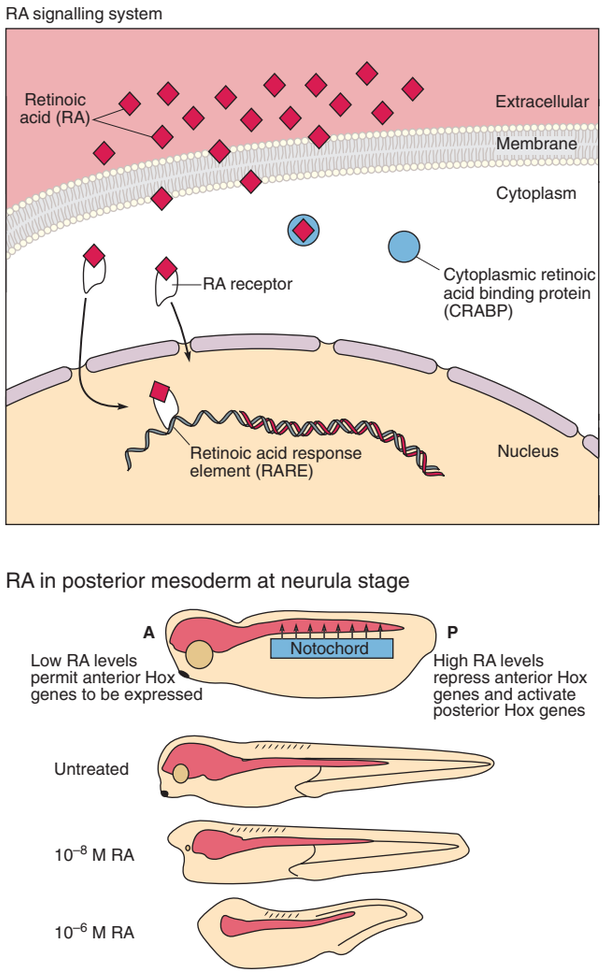

FIGURE 2.8 Retinoic acid signaling is important for the anterior-posterior pattern of *Hox* gene expression. RA crosses the cell membrane to bind a cytoplasmic receptor. The retinoic acid receptor (RAR) translocates into the nucleus where it can regulate gene expression through interaction with retinoic acid response element (RARE). RA levels are about 10 times higher in the posterior region of *Xenopus* embryos, and RA-treated embryos typically show defects in the anterior parts of the nervous system. When embryos are exposed to increasing concentrations of RA, they fail to develop head structures and the expression of anterior genes is inhibited.

RA concentration, with RA levels about 10 times higher in the posterior region of *Xenopus* embryos. When *Xenopus* embryos are treated with RA, they typically show defects in the anterior parts of the nervous system. When embryos are exposed to increasing concentrations of RA, they fail to develop head structures (Figure 2.8), and the expression of anterior *Hox* genes is inhibited (Durston et al., 1989).

Do the teratogenic effects of RA have anything to do with the control of regional identity in the CNS? In fact, it has been known for some time that retinoic acid can induce the expression of *Hox* genes when added to embryonic stem cells. With low concentrations of RA added to the ES cells, only those *Hox* genes normally expressed in the anterior embryo are expressed, while at progressively higher concentrations of RA, more posteriorly expressed *Hox* genes are expressed in the cell line (Simeone et al., 1991). Targeted deletion of the RARs produces defects similar to those observed from pharmacological manipulation of this pathway (Chambon et al., 1995). Finally, both the *Hoxa1* and the *Hoxb1* promoters have RAREs, and these elements are both necessary and sufficient for the rhombomere-specific pattern of expression of these genes. These facts all point to the importance of RA signaling in hindbrain development, but where does the gradient of RA come from in normal embryos? Early models of gradient formation invoked a highly expressing source of the signal and a declining gradient from the source, possibly "sharpened" by an active degradation mechanism. Evidence from several labs now indicates that the source of the RA is the mesoderm that lies immediately adjacent to the neural tube. The so-called paraxial mesoderm contains enzymes that synthesize the RA, and this then diffuses into the hindbrain neural tube to activate the pattern of *Hox* gene expression. The fact that the nonneural tissue outside the developing nervous system can have such a critical impact in its formation reminds us that the nervous system does not develop in a vacuum, but rather many important aspects of its development rely on interactions with adjacent nonneural tissues.

Overall, the similarity of body segmentation in *Drosophila* and hindbrain rhombomere development in vertebrates has led to a rapid understanding of both processes. However, the development of other regions of the vertebrate nervous system does not rely so heavily on the same mechanisms. Instead, other types of transcription factors control the development of the more anterior regions of the brain. In the next sections we will review how divisions in these other brain regions arise.

SIGNALING MOLECULES THAT PATTERN THE ANTERIOR-POSTERIOR AXIS IN VERTEBRATES: HEADS OR TAILS

The overall organization of the anterior-posterior axis of the nervous system in vertebrates is coupled with earlier events in axis determination and neural induction. As noted in Chapter 1, evidence from

Spemann and others demonstrated that there may be separate "head" and "tail" organizers. This fact suggests that the very early inductive signals for neural development also influence the A-P axis. In a now classic experiment, Nieuwkoop (see Chapter 1) transplanted small pieces of ectodermal tissue from one embryo into a host at various positions along the anterior-posterior axis. In all cases, the transplanted cells developed anterior neural structures. However, when the cells were transplanted in the caudal neural plate, posterior structures, such as spinal cord, also developed. Therefore, he concluded that the initial signal provided by the organizer is to cause ectodermal cells to develop anterior characteristics, known as the "activator," while a second signal is required to transform a portion of this neural tissue into hindbrain and spinal cord, known as the "transformer."

Several more recent lines of evidence are consistent with the activator-transformer hypothesis. For example, the neural inducers that have been identified (e.g., noggin, chordin, follistatin) produce primarily anterior brain structures when added to animal caps (see Chapter 1). Also, as described in Chapter 1, targeted deletion of putative neural inducers, such as the noggin/chordin double knockout mouse, results in headless mice. At the present time, three molecular pathways have been implicated as contributing to the "transformer" activity. As described above, retinoic acid treatment can posteriorize embryos and is almost certainly responsible for the patterning of the hindbrain *Hox* gene expression. Other groups have found that there is an endogenous AP gradient of *wnt/beta-catenin* activity in the embryo, with the highest levels in the posterior of the embryo (Kiecker and Niehrs, 2001).

Several lines of evidence support the hypothesis that development of head structures and brain neural tissue requires the inhibition of not only the BMP signaling pathway, but also the inhibition of the *wnt* pathway (Glinka et al. (1997); Figure 2.9). When dominant-negative *wnt8* was injected into *Xenopus* embryos along with the truncated BMP receptor, complete ectopic axes, including head structures, were formed. In addition, several inhibitors of the *wnt* pathway are expressed in the organizer region. One of the first factors specifically implicated in head induction was called cerberus, after the three-headed dog that guards the gates of Hades in Greek mythology. Injection of cerberus into *Xenopus* embryos causes ectopic head formation without the formation of trunk neural tissue (Baumeester et al., 1996). A second *wnt* inhibitor, known as *frzB*, is a member of a family of proteins that are similar to the receptors for the *wnt* proteins, known as frizzleds. The *frzB* proteins work by binding to the *wnt* proteins and preventing them from binding to their

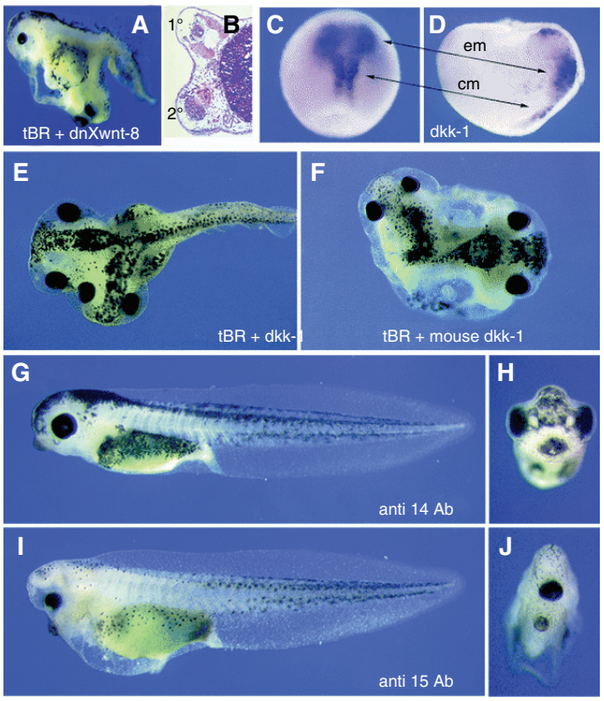

FIGURE 2.9 Heads vs. Tails: the role of Wnt signaling. Antagonism of *Wnt* signaling is important for head induction in frog embryos. A,B. Injection of four-cell embryo with both the truncated BMP receptor (tBR) and a dominant-negative form of *wnt8 (dnXwnt8)* causes frog tadpoles to develop a second head. B shows a section through such a tadpole revealing both the primary and secondary brains. C,D. Expression of *dkk-1* in late gastrulae (stage 12) *Xenopus* embryos. *In situ* hybridization of embryo whole-mount (C) and section (D). Embryos are shown with animal side up, blastopore down. Arrows point to corresponding domains in C and D. The endomesoderm (em) is stained in a wing-shaped pattern, and most posterior expression is in two longitudinal stripes adjacent to the chordamesoderm (cm). E,F. Injection of either *Xenopus* or mouse *Dkk-1* into the blastomeres of a four-cell-stage frog embryo causes an extra head to develop as long as the truncated BMP (tBR) receptor is co-injected. G-J. *Dkk-1* is required for head formation. Stage 9 embryos were injected with antibody (Ab) into the blastocoel and allowed to develop for three days. G,H. Embryos injected with a control (anti-14) antibody show no abnormalities. An anterior view is shown on the right. I,J. Embryos injected with anti-*dkk1* (anti-15) antibody show microcephaly and cyclopia. An anterior view is shown on the right. Note that trunk and tail are unaffected. (Modified from Glinka et al., 1997; Glinka et al., 1998)

signaling receptor. Injection of extra *frzB* into *Xenopus* embryos also causes them to form heads larger than normal. A third *wnt* inhibitor was isolated by a functional screen similar to that described for noggin (see above); in this case, Niehrs and colleagues injected the truncated BMP receptor (tBMPR) along with pools from a cDNA library and looked for genes that would cause complete secondary axes, including heads, only

when co-injected with the tBMPR. They identified a gene that was particularly effective in inducing head structures, *dickkopf*, for the German word meaning big-head or stubborn (Glinka et al., 1998). These three *wnt* inhibitors are reminiscent of the BMP inhibitors described above, in that they are expressed in the organizer region during the time when the inductive interactions are taking place, and they all have head-inducing activity, particularly when combined with a BMP inhibitor (Figure 2.9).

The evidence that there are indeed several putative *wnt* inhibitors in the organizer is good support for the model that a co-inhibition of *wnt* and BMP signals leads to induction of the anterior neural structures, that is, the brain. In fact, the cerberus protein can inhibit both the *wnt* and BMP pathways. Additional support for the model has recently been obtained from studies of mice in which the mouse homolog to *dickkopf, dkk1*, has been deleted via homologous recombination. The mice lacking *dkk1* alone are similar to the compound noggin/chordin knockout mice described above: they lack head and brain structures anterior to the hindbrain (Mukhopadhyay et al., 2001; Figure 2.10). Synergy between the BMP antagonist, noggin, and the *wnt* antagonist, *dkk1*, can be seen by producing mice with a single allele of each of these genes. Although the loss of a single allele of either of these genes has no discernible effect on mice, the loss of a single allele of both of these genes causes severe head and brain defects, similar to those animals that have lost both alleles of the *dkk1* gene. Similarly, knocking out the *wnt* inhibitor *dkk1* leads to a headless embryo, and in the zebrafish mutant *masterblind*—where there is a loss of axin, a component of *wnt* inhibitory signaling pathway—no forebrain develops. Taken together, the studies in mice show that the wnt and BMP antagonists work together to bring about the correct induction and pattern of the nervous system.

The third class of molecules that has been proposed as a "transformer" is FGF. FGFs are able to act as neural inducers and in addition are able to induce posterior gene expression in animal caps that have undergone "neural induction" using a BMP antagonist. Although the specific FGF necessary for the endogenous transforming activity is not known, several members of this family are expressed in early development. Although the relative contributions of FGF, *wnt*, and RA signaling pathways for A-P axis specification in the brain are not clear, work by Kudoh et al. (2003) indicates that these factors may all converge on a common pathway. Both FGF and *wnt* signals suppress expression of *cyp26*, an enzyme involved in retinoic acid metabolism. Without this enzyme, the levels of RA rise in the anterior of the embryo, which could lead to posteriorization.

How do these signals—FGF, RA, and *wnt*—direct the development of the different brain regions? As noted above, the *Hox* genes are critical in the development of rhombomere identity; however, two other homeodomain transcription factors—*Otx2* and *Gbx2*—are necessary for a more fundamental division of the brain, the division between the hindbrain and the forebrain (Joyner et al., 2000). At late gastrula/early neural plate stages in the frog, one can already see these genes expressed in domains adjacent to one another: *Gbx2*-expressing cells extend from the poste-

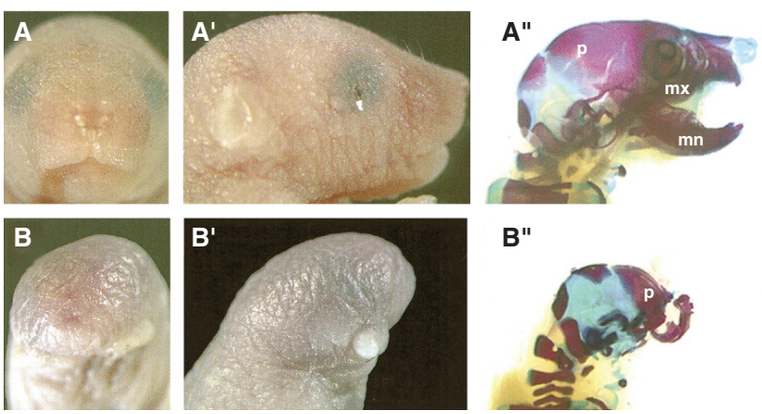

FIGURE 2.10 *Dkk1* and noggin cooperate in head induction. Mice in which one allele for the genes for both *Dkk1* and *Nog* have been deleted have severe head defects. Frontal (*A*,*B*) and lateral (*A*′,*B*′) views of wild-type (*A*,*A*′) and mutant (*B*,*B*′), newborn animals. Lateral view of skeletal preparations from wild-type (*A*″) and severe mutant (*B*″) newborn heads reveal loss of maxillar (mx), mandibular (mn), and other bones anterior to the parietal bone (p). (Modified from del Barco Barrantes et al., 2003)

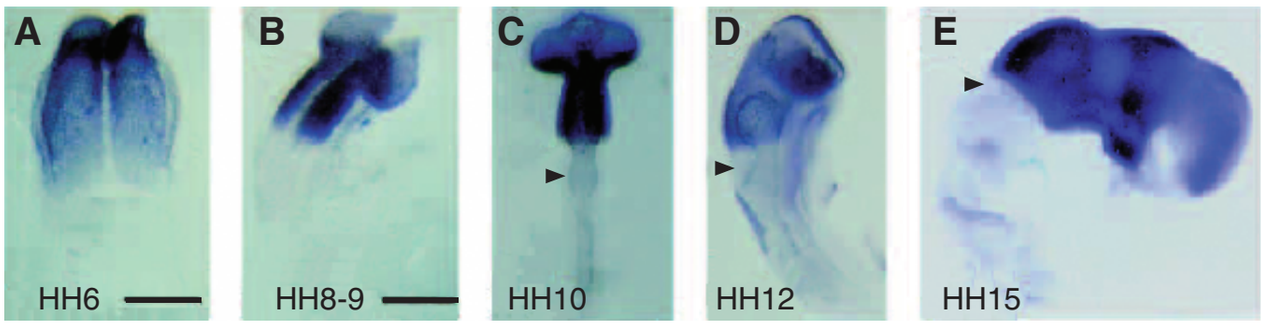

FIGURE 2.11 Expression of *otx2* reflects the basic division between the rostral brain and the hindbrain and spinal cord. *Otx2* expression at various stages of embryonic development in the chick brain. *Otx2* is expressed in the anterior neural plate (A) and remains expressed in most of the brain throughout development (B–E). The arrowhead points to the midbrain–hindbrain boundary. (From Millet et al., 1996)

rior end of the brain to the midbrain/hindbrain border, while *Otx2* has the complementary pattern of expression, from the midbrain/hindbrain border to the anterior-most part of the brain (Figure 2.11). Direct evidence that shows these genes are critical for this fundamental division of the CNS into anterior and posterior compartments come from mouse gene targeting experiments. Deletion of the *Otx2* gene in mice results in animals without a brain anterior to rhombomere 3 (Figure 2.12; Matsuo et al., 1995; Acampora et al., 1995). In mice without the *Gbx2* gene, the converse result is observed: the mice lack the hindbrain region (Millet et al., 1999; Wassarman et al., 1997). These genes are initially induced in this region by another type of transcription factor, known as *Xiro*. One current model is that *Xiro* activates both *Otx2* and *Gbx2*, which then cross-repress one another to create a sharp border between them (Glavic et al., 2002). This type of cross repression of transcription factors is a widely used mechanism for the generation of distinct boundaries between expression domains in the embryo. As we shall see in the next section, the midbrain/hindbrain boundary becomes an important organizing center in its own right.

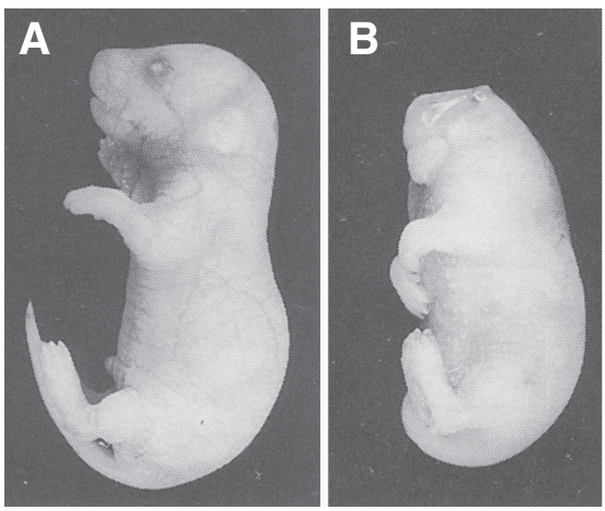

FIGURE 2.12 *Otx2* is required for the formation of the mouse head. A dramatic illustration of the importance of the *otx2* gene in the development of the mouse forebrain and rostral head. If the gene is deleted using homologous recombination, embryos without either allele of the gene fail to develop brain regions rostral to rhombomere 3, a condition known as anencephaly. Since many of the bones and muscles of the head are derived from neural crest, which also fails to form in these animals, the animals lack most of the head in addition to the loss of the brain. (From Matsuo et al., 1995)

ORGANIZING CENTERS IN THE DEVELOPING BRAIN

The division between the metencephalon and the mesencephalon appears to be a fundamental one. This boundary is a major neuroanatomical division of the mature brain as well; the metencephalon gives rise to the cerebellum, and the mesencephalon gives rise to the midbrain (superior and inferior colliculi) (Figure 2.13). But in addition to the important neural structures derived from this region, the midbrain/hindbrain border (or mesencephalon/metencephalon border) has a special developmental function. Like the Spemann "organizer" of the gastrulating embryo, the midbrain/hindbrain border expresses signaling molecules that have an important organizing influence on the development of the adjacent regions of the neuroepithelium.

The idea that specific regions of the neural tube act as organizing centers for patterning adjacent regions was

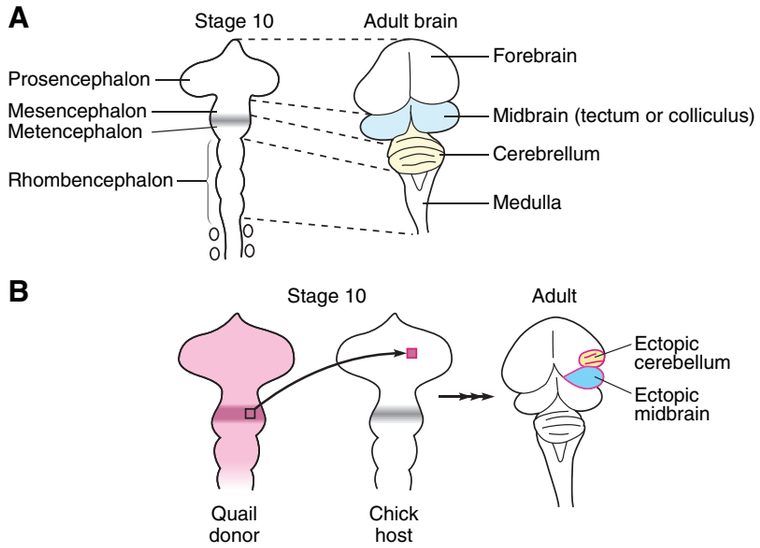

FIGURE 2.13 A signaling center at the midbrain-hindbrain (mesencephalon-metencephalon) boundary organizes this region of the brain. A. During normal development, the region of the midbrain-hindbrain junction expresses the homeodomain transcription factor *engrailed* (red), and this region of the neural tube contains the progenitors of the midbrain (tectum) and the cerebellum. B. To determine whether these parts of the neural tube were restricted in their potential at this time in development, Alvarado-Mallart et al. transplanted a small piece of the quail metencephalon (red) to the forebrain of a similarly staged chick embryo. Cerebellum still developed from the metencephalon transplants, but in addition, the transplanted tissue had induced a new mesencephalon to develop from the adjacent forebrain neural tube cells.

first put on a firm molecular basis through studies of the midbrain/hindbrain border. In a series of experiments designed to test the state of commitment of this part of the neural tube, Alvarado-Mallart and colleagues transplanted small pieces of the neuroepithelium from the midbrain/hindbrain border of chick embryos to similarly staged quail embryos (Alvarado-Mallart, 1993). Grafting between these two species allows the investigator to follow the fate of the transplanted cells. Although the chick and quail cells behave similarly and integrate well together in the tissues, molecular and histological markers can be used to tell them apart after histological processing. When the presumptive metencephalon region was transplanted from a quail to the metencephalon of a chick embryo, the transplanted cells developed as cerebellum. When cells from the mesencephalon were transplanted to a corresponding region of the chick embryo, the cells developed into midbrain structures, like the optic tectum (or superior colliculus). However, when cells from the metencephalon were transplanted to the forebrain, not only did cerebellum still develop from the metencephalon transplants (Figure 2.13) but, surprisingly, the transplanted tissue "induced" a new mesencephalon to develop in the forebrain. In other words, the small piece of hindbrain neural tube was able to re-pattern the more anterior regions of the neural tube to adopt more posterior identities. This experiment is reminiscent of the organizer transplant of Spemann, in that a small region of specialized tissue is able to re-pattern the surrounding neuroepithelium when transplanted.

Several important signaling molecules have been localized to this region and are now known to play a key role in these patterning activities, including *wnt1, engrailed (en1),* and FGF*8*. A member of the *wnt* gene family, *wnt1*, is expressed in this region (Figure 2.15), and when this gene is deleted in mice, the animals lose most of the midbrain and cerebellum (McMahon and Bradley, 1990). One of the earliest observed defects in these animals is the loss of expression of a transcription factor, *engrailed-1* (or *en1*), which is normally expressed in the region of the mesencephalon-metencephalon boundary. The expression of *en1* in this region has also been shown to be critical for normal development of midbrain and hindbrain structures. Mice homozygous for a targeted deletion in the *en1* gene are missing most of the cerebellum and the midbrain similar to the *wnt1*-deficient mice (Wurst et al., 1994). *en1* and *wnt1* were first identified in *Drosophila* segmentation mutants; when either of these genes is defective in flies,

the animals have defects in segmentation. Moreover, in *Drosophila* the homologous gene for *wnt1*, *wingless*, is required for maintaining the expression of the *Drosophila engrailed* gene at the segment boundaries. Thus, the midbrain–hindbrain boundary is another example where the same basic mechanisms as those used in segmentation in *Drosophila* create differences and boundaries in the brain. In addition to the *wnt* and *engrailed* patterning system, the midbrain–hindbrain junction also expresses another key signaling molecule, *FGF8,* a receptor tyrosine kinase ligand. *FGF8* is necessary for both setting up this boundary and maintaining it, since mice deficient in *fgf8* show defects in cerebellar and midbrain development similar to the *Wnt1* and *En1* knockout animals (e.g., Meyers et al., 1998). *fgf8, En1*, and *wnt1* seem to be in an interconnected network, since deleting any one of them affects the expression of the other two. *fgf8*'s role in patterning the tissue around the mes-met boundary was demonstrated in a remarkable experiment; Crossley et al. (1996) placed a bead coated with *fgf8* protein onto a more anterior region of the neural tube and found that this molecule was sufficient to induce the repatterning of these anterior tissues into midbrain and hindbrain structures (Figure 2.14) (Crossley et al., 1996). Thus, the *fgf8* produced by midbrain/hindbrain acts like an "organizer" for the midbrain and hindbrain.

The model of how the midbrain–hindbrain signaling center arises described above can thus be extended as follows (Figure 2.16). *Xiro* activates both *Otx2* and *Gbx2* in this region of the developing CNS. *Gbx2* and *Otx2* cross inhibit one another, and it is at this point of inhibition that *fgf8* is expressed (Glavic et al., 2002). The interaction between *Otx2* and *Gbx2* maintains *fgf8* expression, and *fgf8* induces *engrailed* in those cells that express both *Xiro* and *Otx2*. Through these cross-regulatory loops between cells, the border is initially set up and maintained through development (Rhinn and Brand, 2001). The *FGF8* produced by this region then goes on to regulate growth of the progenitor cells in this region to ultimately produce the brain structures of the midbrain and hindbrain, including the cerebellum and the superior colliculus.

The unique signaling characteristics of the midbrain–hindbrain boundary suggest that such localized organizing centers may be a basic mechanism of brain patterning. There is evidence that other key organizing regions may exist between the dorsal and ventral thalamus and at the anterior pole of the neural tube. Moreover, as development proceeds and the brain expands, new organizers and signaling centers appear to pattern the newly expanded regions. It may be that the appearance of new signaling centers coincides with the expansion of the neuroepithelium past the

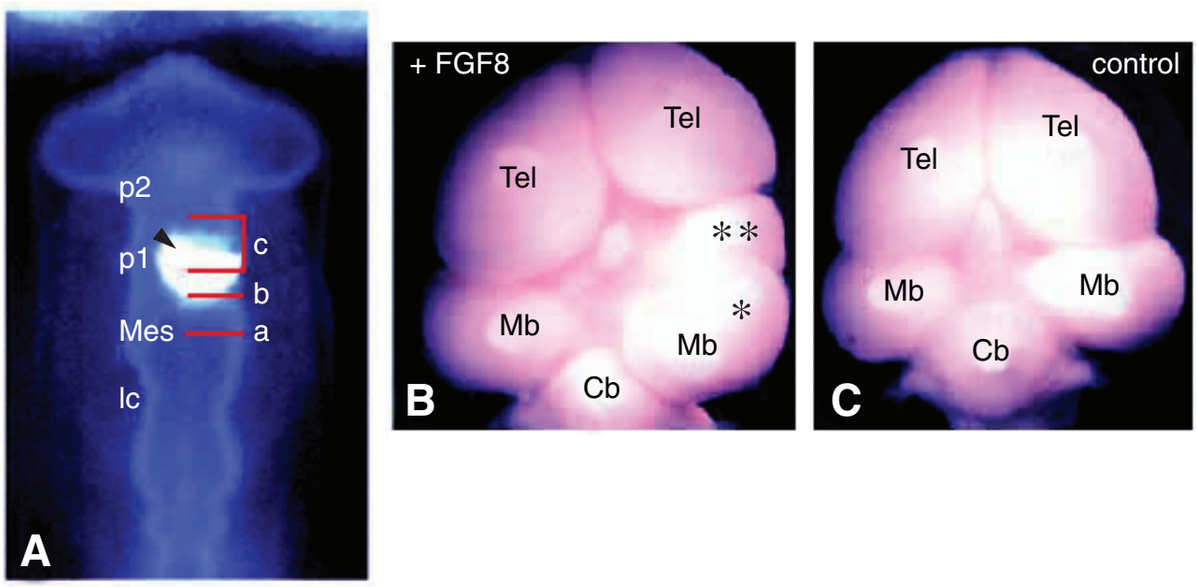

FIGURE 2.14 *FGF8* is a critical signal for the "organizer" activity of the mes-met boundary tissue. (A) Crossley et al. placed a bead (shown in arrow) of *FGF8* onto the telencephalon of the chick embryo and found that this caused a new mes-met boundary to form with a mirror duplicated midbrain (B), similar to the transplant experiment of Alvarado-Mallart. (C) Shows the control animal. (Modified from Martinez et al., 1999)

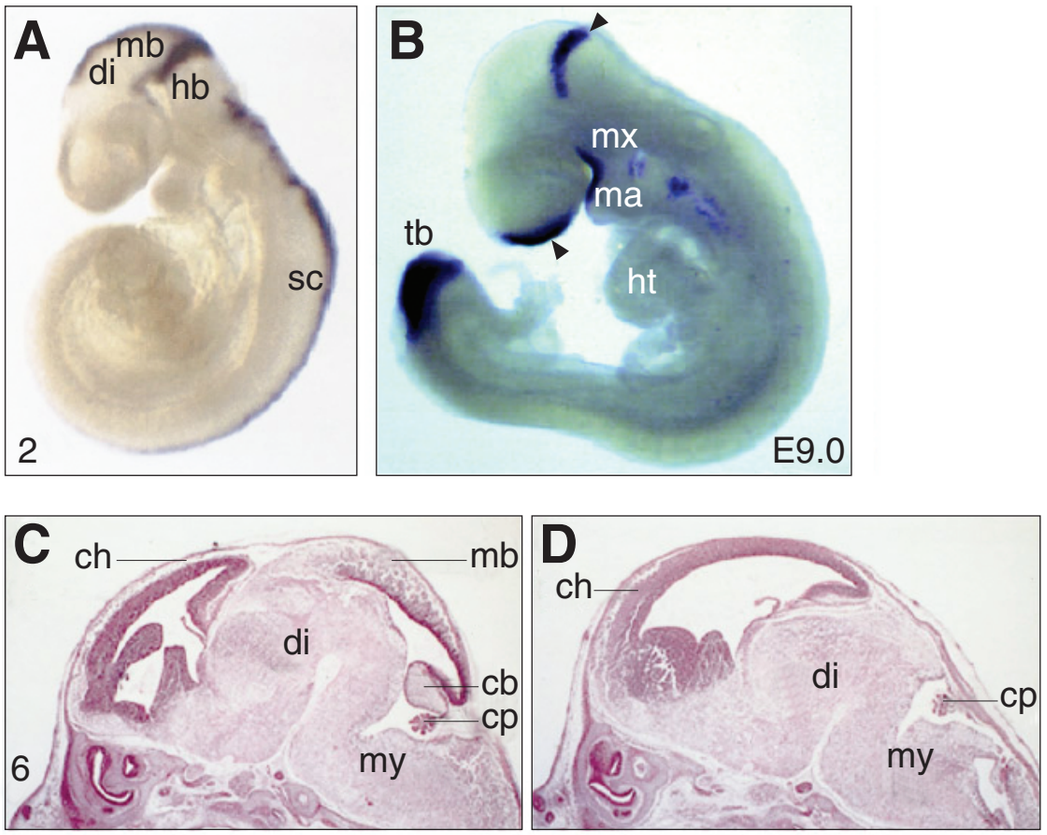

FIGURE 2.15 Several important signaling molecules have been localized to the midbrain-hindbrain boundary, a key signaling center in the brain. *wnt1* (A) *engrailed-1* and *fgf8* (B) form an interconnected network that specifies this boundary and is necessary for the growth of the midbrain and the cerebellum. Deletion of any of these molecules in mice results in a loss of the midbrain and reduction in cerebellar size. A section through the brain of a wild-type embryo is shown in C, while a *wnt1* knockout mouse brain is shown in D. Note the loss of the midbrain (mb) and cerebellum (cb) in the mutant brain. Other structures are normal (ch = cerebral cortex; cp = choroids plexus; di = diencephalon; my = myelencephalon). (A, B, D modified from Danielson et al., 1997; B modified from Crossley and Martin, 1995)

distance over which these molecules can signal. Once the number of cells exceeds the range over which the signal can act, the brain just makes a new signaling source. The same principle seems to be driving the construction of cell phone towers.

FOREBRAIN DEVELOPMENT, PROSOMERES, AND *PAX* GENES

To this point, we have explored how *Hox* genes control the specification of anterior-posterior position in the nervous system. However, *Hox* gene expression stops at the anterior boundary of the metencephalon. Are there similar transcription factors that control positional identity in the rest of the brain? Many other types of homeodomain proteins are expressed in these more anterior regions of both vertebrate and invertebrate embryos, and they perform a role similar to that of *Hox* gene clusters in more caudal segments. Below, we explore the evidence that homeodomain proteins specify the structures that comprise the head and brain.

The most widely held view is that different parts of the brain are generated through the progressive subdivision of initially similar domains. The neural plate begins to show regional differences in the anterior-posterior direction at its formation. Embryologists at the beginning of the last century applied small amounts of dyes to specific parts of developing embryos and found that particular regions of the neural plate are already constrained to produce a particular part of the nervous system. Many embryologists have also used transplantation between species to define the contri-

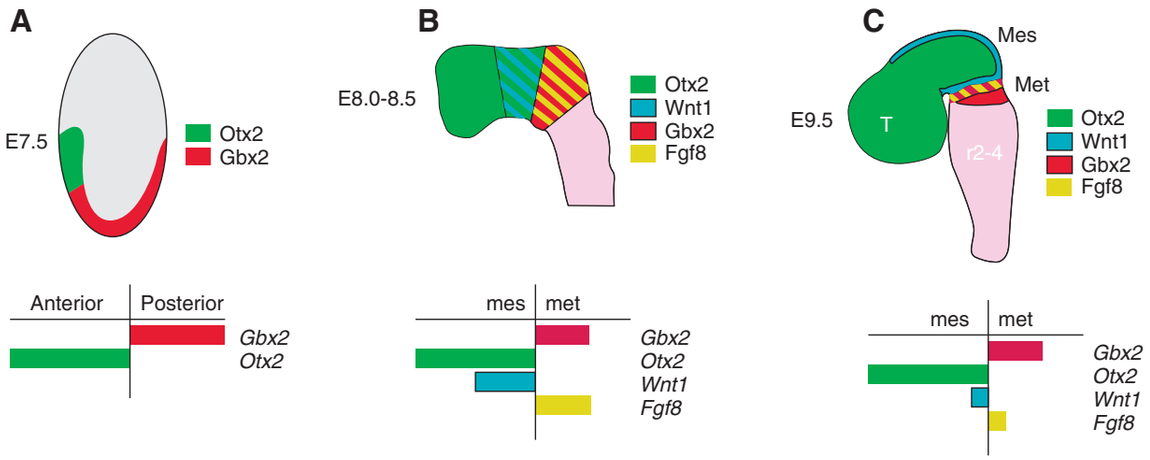

FIGURE 2.16 The model of how the midbrain-hindbrain signaling center arises. A. The initial distinction between the anterior and posterior of the embryonic nervous system is reflected in expression of *otx2* and *gbx2*. C. At the boundary between these two factors, the mes-met boundary forms, and *wnt1, en1,* and *FGF8* are all expressed in this region and act in a regulatory network to maintain their expression and this boundary. (Modified from Joyner et al., 2000)

butions to the mature brain of particular regions of the neural tube. One particularly useful interspecific transplantation paradigm that was developed by Nicole LeDouarin is to transplant tissues between chick embryos and quail embryos, as described in the previous section. Since these species are similar enough at early stages of development, the transplanted cells integrate with the host and continue developing along with them (Figure 2.17). The chick and quail cells can be later distinguished since the quail cells contain a more prominent nucleolus, which can be identified following histological sectioning and processing of the chimeric tissue. More recently, antibodies specific for quail cells have been generated, and these are also useful for identifying the transplanted cells. The combination of vital dyes, cell injections, and chick-quail transplant studies have produced a description or "fate map" of the ultimate fates of the various cells of the embryo. Figure 2.18 shows the fate maps for amphibian (Eagleson and Harris, 1990), avian, and mammalian neural tubes, for the basic forebrain regions that have been derived from these fate-mapping studies. The basic pattern has been elaborated upon to generate the wide diversity of brains that are found in vertebrates.

Although fate-mapping studies provide information about the fate of the different neural tube regions, embryologists have also investigated whether the fate of the cells is fixed or can be changed. The goal of these experiments, in general, is to provide a timetable for

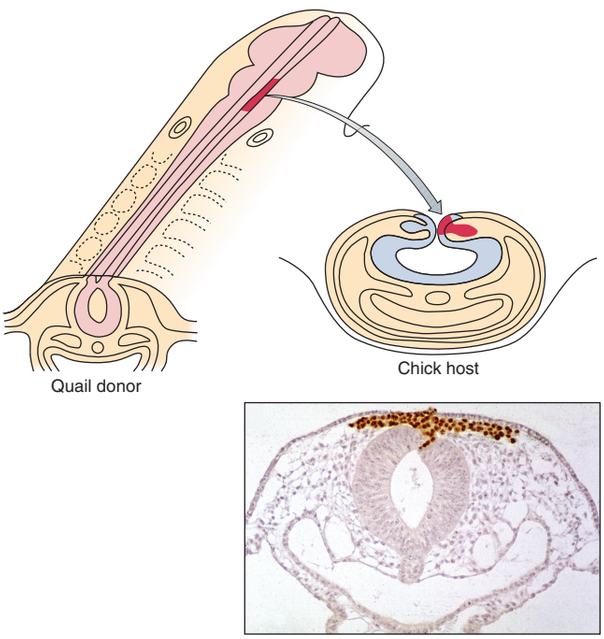

FIGURE 2.17 The interspecific transplantation paradigm was developed by Nicole LeDouarin using chick embryos and quail embryos. Tissue is dissected from quail embryos and then placed into specific regions of live chick embryos. In this case, the dorsal ridge of the neural tube, the region that will give rise to neural crest, is transplanted to a similar region in the chick. The chick and quail are similar enough to allow the quail to contribute to the chick embryo, and the quail cells can be specifically identified with an antibody raised against quail cells (bottom). (Modified from Le Douarin et al., 2004)

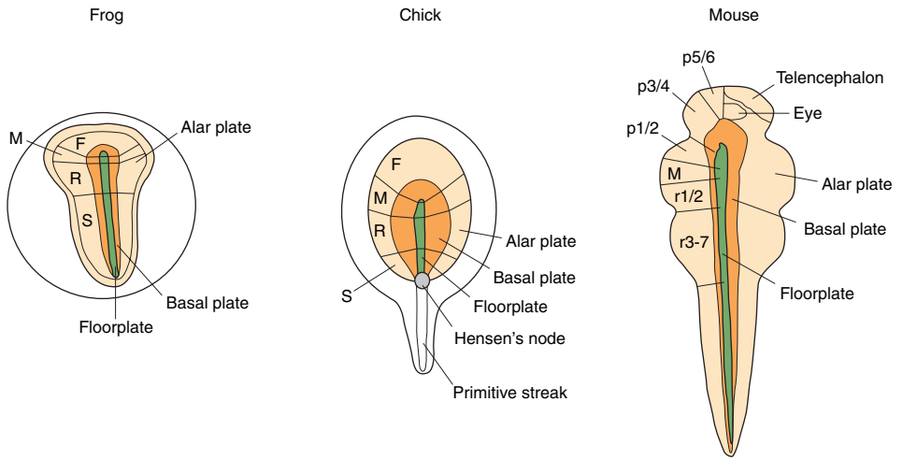

FIGURE 2.18 The fate maps for amphibian, avian, and mammalian neural tubes. The basic forebrain regions are common to all vertebrates; however, the basic pattern has been elaborated upon to generate the wide diversity of brains that are found in vertebrates. The rhombomeric and prosomeric organization of the mouse brain can already be recognized at this early stage by the pattern of expression of certain genes.

understanding the moments in development when molecular mechanisms are actively directing a specific region of the neural tube to its specific fate (i.e., is "specified"). To determine at what point in development this "specification" occurs, pieces of the neural plate are transplanted to ectopic locations in the embryo. If the transplanted cells give rise to a particular brain region, we say that it has already been specified. For example, a piece of the anterior neural plate, near the eye, is transplanted to the presumptive flank of another embryo. After sufficient developmental time has passed, the embryos are analyzed for the type of neural tissue that developed from the graft. In this case the finding is that, as early as late gastrula, a particular region of the neural plate will always give rise to anterior brain, including the eye. This occurs regardless of where the tissue is placed in the host animal. A number of embryologists carried out these types of experiments using various regions of the neural plate as the donor tissue, and the results consistently demonstrate that at some point in development, the cells of the neural plate take on a regional identity that cannot be changed by transplantation to some other place in the embryo. The fact that different regions of the neural plate are already committed to a particular fate has been extended in recent years by the observations that a number of genes are expressed in highly specific regions of the developing nervous system. In many cases the domain of expression of a particular transcription factor corresponds to that region of the neural tube that will ultimately give rise to one of the five brain vesicles, and the gene may continue to be expressed in that brain region throughout its development.

Many embryologists have taken advantage of the patterns in gene expression in the forebrain to gain insight into the basis of its organization. In what has become known as the prosomeric model of forebrain development, it is proposed that there are longitudinal and transverse patterns of gene expression that subdivide the neural tube into a grid of different regional identities (Puelles and Rubenstein, 1993). The expression of some of these genes is shown for the mouse embryo at two different stages of development (Figure 2.19). In many cases, the boundary of expression of a particular gene corresponds closely to the morphological distinctions between the prosomeres. For example, two genes of the *emx* class are expressed in the telencephalon, one in the anterior half of the cerebral hemispheres (*emx1*) and the other in the posterior half of the hemispheres (*emx2*). Thus, the telencephalic lobes can be divided into anterior and posterior segments on the basis of the pattern of expression of these two genes. Analysis of the expression patterns of additional genes has led to the conclusion that the prosencephalon can be subdivided into six prosomeres (Figure 2.19). They are numbered from caudal to rostral, and so prosomere 1 is adjacent to the mesencephalon. *P2* and *P3* subdivide what is traditionally known as the diencephalon, and *P4, P5,* and *P6* subdivide the telencephalon.

While the studies of regional expression of transcription factors present a model of brain organization and evolution, the functional analyses of homeo-

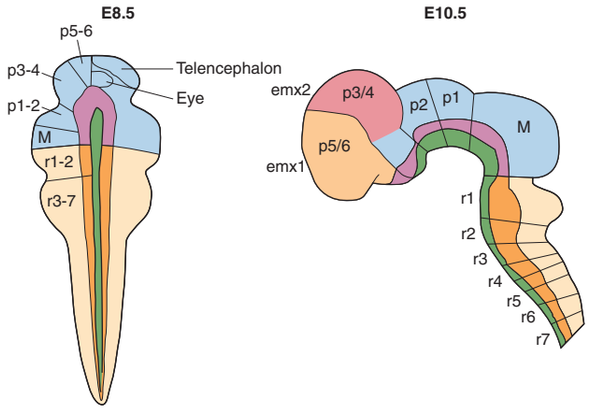

FIGURE 2.19 Prosomeric model of forebrain development; longitudinal and transverse patterns of gene expression that subdivide the neural tube into a grid of regional identities. The expression of some of these genes is shown for the mouse embryo at two different stages of development. Two genes of the emx class are expressed in the telencephalon, one in the anterior half of the cerebral hemispheres *(emx1)* and the other in the posterior half of the hemispheres *(emx2).* Analysis of the expression patterns of additional genes has led to the conclusion that the prosencephalon can be subdivided into six prosomeres. They are numbered from caudal to rostral, and so prosomere 1 is adjacent to the mesencephalon, *P2* and *P3* subdivide what is traditionally known as the diencephalon, and *P4, P5*, and *P6* subdivide the telencephalon.

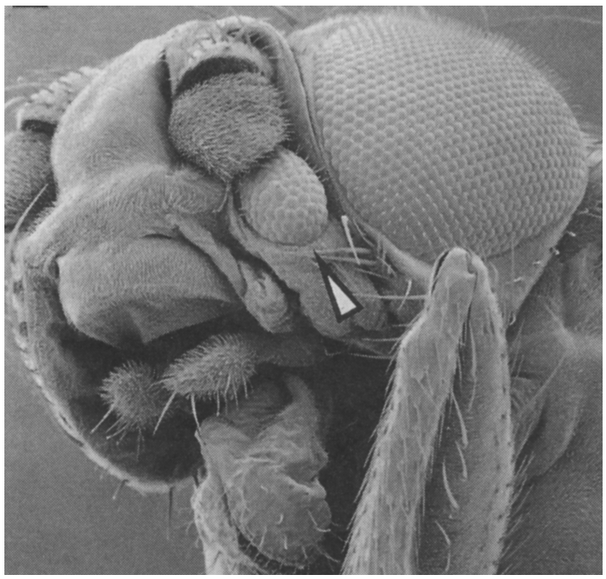

FIGURE 2.20 Ectopic eyes are formed when the *Drosophila pax6* gene—*eyeless*—is misexpressed in other imaginal discs. Halder et al. (1995) misexpressed the *eyeless* gene in the leg disc in the developing fly and found that an ectopic eye was formed in the leg. This remarkable experiment argues for the concept that master control genes organize entire fields, or structures during embryogenesis, possibly by activating tissue specific cascades of transcription factors. (Courtesy of Walter Gehring)

domain factors have yielded remarkable evidence that these molecules are critically involved in defining the regional identity of the anterior brain. There are now many examples of regionally expressed transcription factors that have essential roles in brain development, but only a few will be mentioned. However, one principle that emerges is that several different classes of transcription factors are likely to be important in specifying the positional identity of cells in any particular region of the brain.

A key class of transcription factors that are critical for specifying regional differences in the nervous system are the *pax* genes. These genes have a homeodomain region, and they also have a second conserved domain known as the paired box (named for its sequence homology with the *Drosophila* segmentation gene, *paired*). There are nine different *pax* genes, and all but two, *pax1* and *pax9*, are expressed in the developing nervous system (Chalepakis et al., 1993). Several of these genes are also disrupted in naturally occurring mouse mutations and human congenital syndromes, and the defects observed in these conditions generally correspond to the areas of gene expression. *pax2*, for example, is expressed in the developing optic stalk and the otic vesicle of the embryo, and mutations in *pax2* in mice and humans cause optic nerve abnormalities, known as colobomas.

Perhaps the most striking example of *Pax* gene regulation of regional differentiation in the nervous system comes from the studies of *Pax6*. This gene is expressed early in the development of the eye, when this region of the neural plate is committed to giving rise to retinal tissue. Humans with a heterozygous disruption of this gene exhibit abnormalities in eye development, causing a condition known as aniridia (a lack of formation of the iris). In mice and humans with a homozygous disruption of this gene, the eyes fail to develop past the initial optic vesicle stage. A homologous gene has also been identified in *Drosophila* (as well as many other organisms), and mutations in this gene also disrupt eye formation in flies. And even more surprising, when this gene is misexpressed at inappropriate positions in the embryo, ectopic eyes are induced (Halder et al., 1995 (Figure 2.20). The ability of a single gene to direct the development of an entire sensory organ like the eye is striking, and while in flies the *Pax* genes act as if they are at the top of a hierarchy, and can be thought of as coordinating the signals and genes necessary to organize a "field" of the embryo's development, the situation in vertebrates is considerably more complex. The *Pax6* gene is one of several transcription factors that are expressed in the

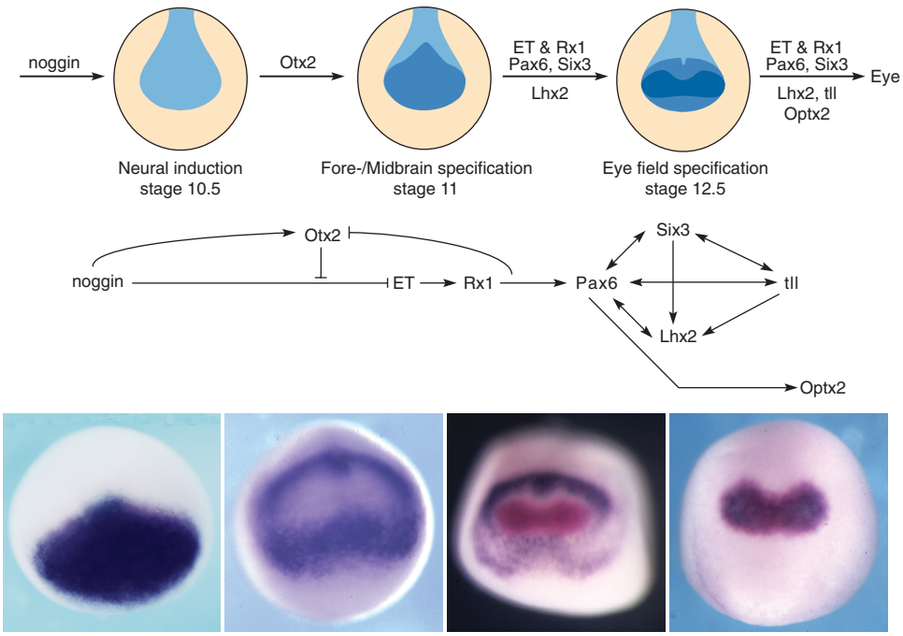

FIGURE 2.21 Summary model of eye field induction in the anterior neural plate. The top of the figure shows dorsal views of the neural plate of *Xenopus* embryos at successively later stages of development from left to right. Light blue indicates the neural plate, blue shows the area of *Otx2* expression, and dark blue represents the eye field. The diagram shows the complex relationships among the eye-determining transcription factors, including *pax6, Rx1, Lhx2, Six3, Otx2,* and *tll*. These genes act together to coordinate eye development in this specific region of the neural plate. The bottom panels show examples of *in situ* hybridizations for several eye transcription factors to show their specific patterns of expression in the presumptive eye-forming region of the embryo. (Modified from Zuber et al., 2003)

eye field, the region of neural plate fated to become the eye. Each of these factors is necessary for specification and growth of the eye. Mutations in any one of these transcription factors, including *pax6, Rx1, Lhx2, Six3, ET*, all have devastating effects on the development of the eye. Whereas overexpression of some of these factors on their own can cause the formation of ectopic eyes in *Xenopus* frogs, overexpression "cocktails" of several of the factors together have much more potency in inducing ectopic eyes (Zuber et al., 2003) (Figure 2.21). Thus, several different transcription factors may be necessary to control the expression of genes necessary for development of such a complex sensory organ as the eye.

DORSAL-VENTRAL POLARITY IN THE NEURAL TUBE

The early neural tube consists only of undifferentiated neural and glial progenitor cells. The neural tube is essentially a closed system, and the brain vesicles and developing spinal cord are fluid filled chambers. The surface of the tube, adjacent to the lumen, is known as the ventricular surface, since eventually the lumen of the neural tube goes on to form the ventricular system of the mature brain. The progenitor cells for neurons and glia of the CNS have a simple bipolar morphology and initially span the width of the neural tube. As these cells undergo mitotic divisions, they typically go through the M-phase of the cell cycle at the ventricular surface. The postmitotic immature neurons generated from the progenitor cells migrate away from the ventricular zone toward the margin of the spinal cord to form the mantle layer (see Chapter 3).

At the neural plate stage, several mechanisms are set in motion that will define the overall organization of the neural tube. First, the most ventral part of the neural tube becomes flattened into a distinct "floorplate." Second, the most dorsal aspect of the neural tube develops into a tissue known as the roofplate. Third, a distinct fissure, the *sulcus limitans*, forms between the dorsal and ventral parts of the neural tube along most of its length (Figure 2.22). These structures are an early sign that the neural tube is differentiating

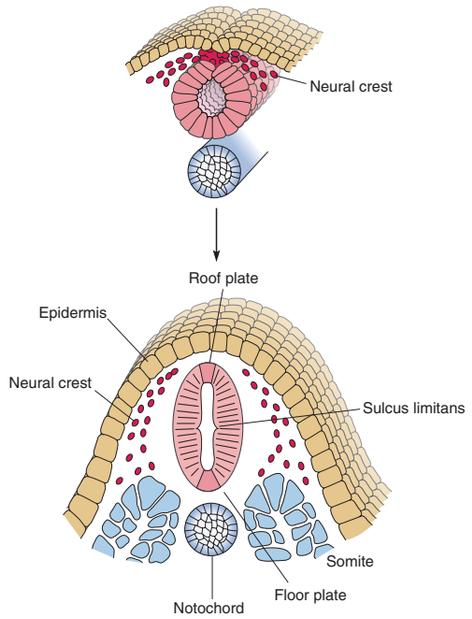

FIGURE 2.22 The overall organization of the neural tube emerges soon after closure. The most ventral part of the neural tube becomes flattened into a distinct "floorplate." The most dorsal aspect of the neural tube develops into a tissue known as the roof plate. A distinct fissure, the *sulcus limitans*, forms between the dorsal and ventral parts of the neural tube along most of its length.

along the dorsal-ventral axis. Later, the neural tube will become even more polarized along this axis; in the ventral part of the tube, motor neurons will begin to arise, while in the dorsal part, the sensory neurons form. Experiments over many years have led to the conclusion that the distinct polarity of the neural tube arises largely because of the interaction between the surrounding nonneural tissue and the neural tube. Experiments during the early part of the twentieth century by Holtfreter demonstrated that the basic dorsal–ventral polarity of the neural tube was dependent on an adjacent, nonneural structure, called the notochord. Isolation of the neural tube from the surrounding tissues resulted in an undifferentiated tube, without obvious motoneuronal differentiation in the ventral tube. However, when he transplanted a new notochord to a more dorsal location, this induced a second floorplate (Figure 2.23) and motoneuron differentiation in the dorsal neural tube. Thus, the notochord is both necessary and sufficient for the development of the dorsal–ventral axis of the spinal cord.

The studies that led to identification of the signals that control dorsal-ventral polarity in the developing spinal cord relied on the use of many molecular markers of cell identity that were obviously not around at the time Holtfreter was doing his experiments. These genes include the *pax* class of transcription factors discussed in the previous section, as well as a variety of other genes that are restricted to particular populations of both differentiated and/or undifferentiated cells within the spinal cord. The expression of some of the critical genes that define the dorsal-ventral polarity of the spinal cord are summarized in Figure 2.24. To track down the polarity signal released by mesoderm, a cell culture system was devised in which the notochord and the neural tube were co-cultured in collagen gels. The signal was first shown to be diffusible, since pieces of notochord could induce floorplate without touching the neural tube. In addition, the expression of motoneuron-specific genes, such as choline acetyltransferase, was also shown to depend on the notochord. A clue to the identity of the factor was uncovered in a rather roundabout manner. A crucial clue about the identity of the notochord signal would, again, come from *Drosophila.* During a large screen for developmental mutants in the fruit fly (Nusslein-Volhard and Wieschaus, 1980), a severely deformed mutant was found, named *hedgehog* for its truncated appearance. Subsequent cloning of the gene showed that this molecule resembled a secreted protein.

The link between hedgehog and the notochord-signaling molecule began with the identification of the mammalian homolog, called *Sonic hedgehog* (*Shh*). *Shh* is expressed initially in the notochord at the time when the dorsal–ventral axis of the neural tube is being specified (Roelink et al., 1994). Shortly after this time, the expression of sonic hedgehog begins in the differentiating ventral neural tube, leading to floorplate. This expression pattern is consistent with the transplantation experiments of Harrison and more recently of Tom Jessell and co-workers; both found that initially the ventralizing signal arises from the notochord, but soon after it is also found in the floorplate. To determine whether *Shh* was indeed the inducer of dorso-ventral polarity in the spinal cord, a small aggregate of *Shh* expressing Cos cells was placed next to the neural tube. The *Shh* released from these cells was sufficient to induce a second floorplate, as well as other genes normally expressed in the ventral neural tube. In further experiments, simply adding recombinant *Shh* protein to explants of neural tube was sufficient to induce them to differentiate as ventral neural tissues, including floorplate and motor neurons (Figure 2.25). These experiments thus show that *Shh* is sufficient to ventralize the neural tube during development. Two additional results show that *Shh* is required during normal development to specify the dorsal–ventral axis

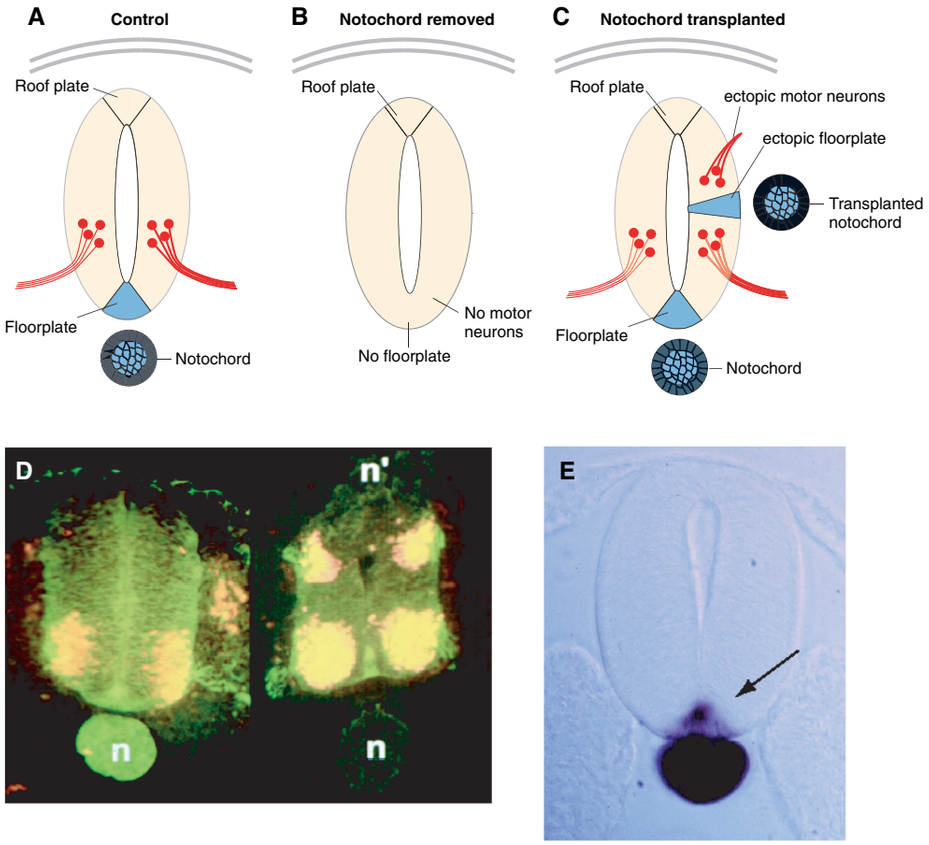

FIGURE 2.23 Differentiation in the neural tube is dependent on factors derived from adjacent, nonneural tissues. The diagrams at the top of the figure show that if the notochord, a mesodermally derived structure, is removed prior to neural tube closure, the neural tube fails to display characteristics of ventral differentiation, such as the development of the floorplate (blue) and the spinal motoneurons (red). This shows that the notochord is necessary for the development of ventral neural tube fates. If an additional notochord is transplanted to the lateral part of the neural tube at this same time in embryogenesis, a new floorplate is induced adjacent to the transplanted notochord. New motoneurons are also induced to form adjacent to the ectopic floorplate. Thus, the notochord is sufficient to specify ventral cell fates. In the lower panels, the experiment diagrammed at the top, the transplantation of an extra notochord, is shown next to a normal neural tube labeled with a marker for motorneurons. The extra notochord is labeled as *n'*. In the lower right, the expression of *sonic hedgehog* in the notochord and floorplate (arrow) of the neural tube is shown. (B, C, and D courtesy of Henk Roelink)

of the neural tube. First, antibodies raised against *Shh* will block the differentiation of floorplate and motor neurons when added to neural tube explants. Second, targeted deletion of the *Shh* gene in mice results in the failure of the development of the ventral cell types in the spinal cord (see Chapter 4).

In addition to its role in the ventralization of the neural tube, *Shh* is also expressed in the more anterior regions of the body axis immediately subjacent to the neural tube, in what is known as the prechordal mesoderm. Here the function of *Shh* is similar to that of the notochord and floorplate: it serves to induce ventral differentiation in the forebrain. In the forebrain, the growth of the different brain vesicles gives rise to complex anatomy, and so the induction of ventral forebrain is critical for a number of subsequent morphogenetic events. Consequently, the loss of *Shh* signaling in the prechordal mesoderm produces dramatic phenotypic changes in embryos and the resulting animals. One particularly striking phenotype that arises from the disruption of *Shh* in embryogenesis is cyclopia (Roessler et al., 1997). The eyes normally form from paired evaginations of the ventral diencephalon (see above). However, in the neural plate, the eye field is initially continuous across the midline and is split into two by the inhibition of eye-forming potential by *Shh*

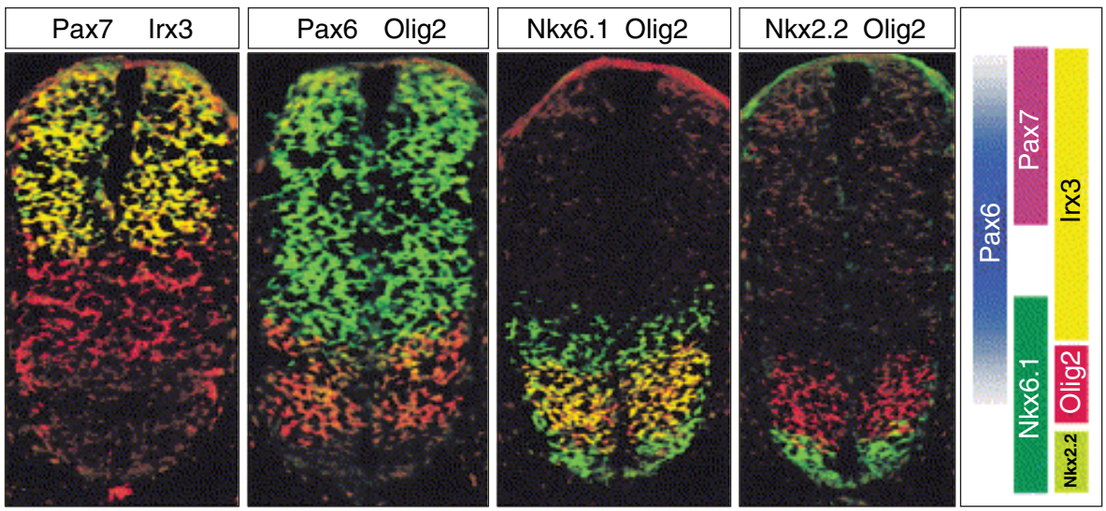

FIGURE 2.24 Several genes are expressed in restricted domains in the developing spinal cord; these have served as useful markers for positional identity of cells in this region of the nervous system. *Pax7, Irx3,* and *pax6* are all expressed in the intermediate and dorsal regions of the neural tube, while *nkx2.2, olig2,* and *nkx6.1* are all expressed in the ventral neural tube. Markers like these and others allowed Jessell and colleagues to dissect the signals controlling the identity of the different types of neurons in the spinal cord (see also Chapter 4). (From Wichtele et al., 2002)

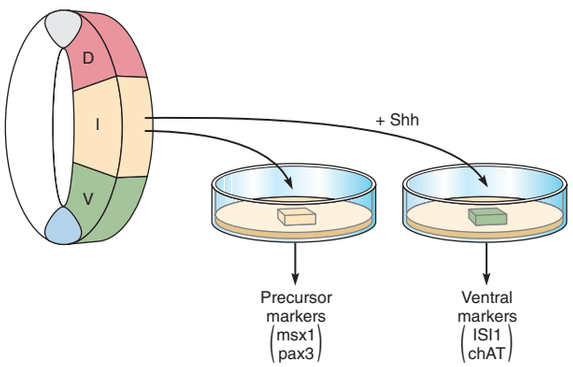

FIGURE 2.25 A cell culture system in which the notochord and the neural tube were co-cultured in collagen gels was used to find the polarity signal released by mesoderm. The signal was first shown to be diffusible since pieces of notochord could induce floorplate without touching the neural tube. Simply adding recombinant *sonic hedgehog* protein to explants of neural tube was sufficient to induce them to differentiate as ventral neural tissues, including floorplate and motor neurons. The dorsal-ventral polarity of the neural tube is controlled in part by a signaling molecule secreted by the mesodermally derived notochord. In the normal embryo, the notochord lies just ventral to the neural tube. The cells at the ventral-most part of the neural tube develop a distinct identity and morphology, and are known as the floorplate (blue). The from embryos at the time of neural plate formation in chick embryos, no floorplate develops, and the neural tube fails to develop motoneurons, or other features of its normal dorsal-ventral polarity. By contrast, if an additional notochord is transplanted to a more lateral position adjacent to the neural tube, an additional floorplate develops, and motoneurons are induced to form adjacent to the new floorplate.

from the prechordal mesoderm. *Shh* represses *Pax6* at the midline and induces *pax2*. *pax6* and *pax2* cross repress, creating a sharp border between developing retinal fields (*Pax6*) and optic stalk region (*Pax2*) that separate the developing retinas. When this signal is interrupted, the eye field remains continuous and a single eye forms in the midline. The subsequent elaboration of the forebrain depends on correct midline development, and so the lack of *Shh* disrupts later stages of brain development as well, leading to a condition known as holoprosencephaly.

The mechanism by which *Shh* induces ventral differentiation of the neural tube involves several interesting signaling steps. In both *Drosophila* and vertebrates, the hedgehog proteins undergo autoproteolysis to generate an amino-terminal fragment that is associated with the cell surface and a freely diffusible carboxyl-terminal fragment. The amino-terminal fragment is sufficient to elicit ventral differentiation as evidenced by floorplate and motoneuron differentiation. Since floorplate differentiation occurs at higher doses of recombinant *Shh* and motoneuron differentiation at lower doses of *Shh*, it has been proposed that a gradient of *Shh* from the notochord and floorplate patterns the neural tube into these two alternate fates. More will be said on this topic in Chapter 4.

Although the experiments described with *Shh* indicate that this molecule can have a profound effect in ventral patterning of the neural tube, more recent

studies have shown that it is not the only factor with this capability. As we noted earlier in this section, retinoic acid is also secreted from the mesoderm and has effects in the neural tube, specifically in the developing hindbrain. Studies by Novitch et al. (2003) have found that RA, along with FGF, can almost completely replace the *Shh* signal and restore ventral development to tissue without any detectable *Shh.* This result, along with the finding that elimination of a downstream effector of *Shh* signaling, the *Gli* transcription factor, from mice can nearly completely rescue ventral development in the *Shh*-deficient mice (Litingtung and Chiang, 2000), indicates that *Shh* may be only one of several redundant molecular signals that pattern the ventral axis of the neural tube. As we saw for neural induction, a multiplicity of partly overlapping signals and transcription factors are responsible for the cellular diversity we know as pattern in the nervous system.

DORSAL NEURAL TUBE AND NEURAL CREST

The experiments of Harrison and others showed that removal of the notochord resulted in a neural tube without much dorso-ventral polarity. This implies that the dorsal neural tube is in some way the default condition, whereas the ventral structures require an additional signal to develop their fates. However, in the last few years it has become apparent that the dorsal neural tube also requires signals for its appropriate development. Before the neural tube closes, the future dorsal neural tube is continuous with the adjacent ectodermal cells. As the dorsal neural tube closes, the neural crest forms at the point of fusion of the neural tube margins. Thus, the neural crest is, in some sense, the most dorsal derivative of the neural tube, and has often been used as an indicator of dorsal differentiation. In addition, several genes specifically expressed in the dorsal neural tube at these early stages of development are critical for the specification of neural crest (e.g., *slug* and *snail*).

After extensive migration, the neural crest gives rise to an array of different tissues. In the trunk, the neural crest gives rise to the cells of the peripheral nervous system, including the neurons and glia of the sensory and autonomic ganglia, the Schwann cells surrounding all peripheral nerves, and the neurons of the gastric mucosal plexus. Several other cell types, including pigment cells, chromatophores, and smooth muscle cells, arise from the trunk neural crest. Neural crest also forms in the cranial regions, and here it contributes to most of the structures in the head. Most of the mesenchyme in the head, including that which forms the visceral skeleton and the bones of the skull, is derived from neural crest. The neurons and glia of several cranial ganglia, like the trigeminal sensory ganglia, the vestibulo-cochlear ganglia, and the autonomic ganglia in the head, are also derived largely from the progeny of the neural crest as well as from the cranial placodes. These placodes that give rise to the nose, the lens of the eye, the otic vesicle, and components of cranial sensory ganglia form a ring around the anterior edge of the neural plate and may be considered as a kind of anterior extension of the neural crest.

Because of the extensive migration of the neural crest cells, and the great diversity of the tissues and cell types to which neural crest cells can contribute, the neural crest has been studied extensively as a model for these aspects of nervous system development. In the next sections we will review what is known about the origin of the neural crest and the factors that control the initial aspects of its differentiation. Chapter 3 will detail additional studies of the factors that control neural crest migration, and Chapter 4 will deal with the cellular determination of various crest derivatives.

Classically, the neural crest has been thought to arise from the cells that form at the fusion of the neural folds when they become the neural tube. Vogt, using vital dyes to fate-map the different parts of the amphibian embryo, found that most of the neural crest forms from a narrow stripe of ectodermal cells at the junction between the neural plate and the epidermis. Subsequent studies using more sophisticated techniques have expanded this view. Le Douarin and her colleagues have extensively used the chick-quail chimera system described above to track the fate of the neural crest that arises from the different regions along the neuraxis to show the different types of tissues that are generated from different rostral-caudal regions (Figure 2.26). Bronner-Fraser and Fraser (1991) used single-cell injections to track the lineages of individual crest cells prior to their migration. The injected cells went on to divide, and they retained their lineage marker for several cell divisions. Many of the labeled cells went on to contribute to the tissues described above as the normal neural crest derivatives; however, some of the labeled cells that contributed to the neural crest also had progeny that populated the neural tube and the epidermis. Thus, although most of the cells in the neural crest field at the neural plate stage of development normally develop into neural crest, they are not restricted to this lineage. In addition, although in many embryos, the neural crest develops at the fusion of the neural folds, there are regions of the neuraxis in some species that do not form by the rolling of the neural plate. For example, in the fish, the neural tube

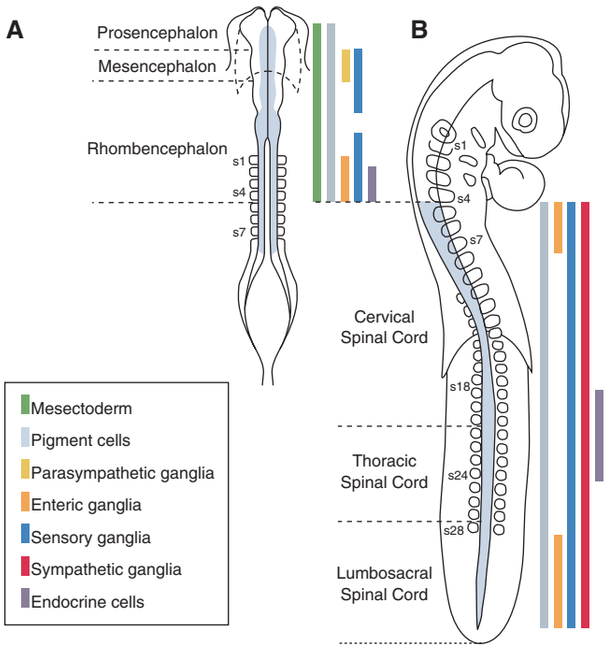

FIGURE 2.26 The fate map of neural crest in the chick embryo. Various types of tissues, including pigment cells, sensory ganglia, and endocrine cells, are derived from the neural crest. The cells migrating from the various positions along the neural tube give rise to different tissues; for example, the sympathetic ganglia arise from the neural crest of the trunk, but not from the head. Similarly, the parasympathetic ganglia arise from the neural crest of the head but not from the crest that migrates from most trunk regions. (Reproduced from Le Douarin et al., 2004)

forms first as a thickening of the neurectoderm, known as the neural keel, and tube formation occurs later by a process of cavitation, but the neural crest still forms from the lateral edges of the plate. Additional recent studies have also shown that although most of the neural crest normally arises from the lateral edges of the neural plate, there is a late-migrating population of crest cells that are derived from the neural tube.

The first experimental studies to indicate that the induction of the neural crest may involve some of the same factors as those responsible for neural induction were those of Raven and Kloos (1945). They found that neural crest was induced from ectoderm by lateral pieces of the archenteron roof, whereas neural tube was induced by medial pieces, such as the presumptive notochord. Similar results led Dalq (1941) to propose that a concentration gradient of a particular organizing substance originating in the midline tissue of the archenteron roof could set up medial-lateral distinctions across the neural plate—"the median strip of the archenteron roof, supposedly rich in organisine, would induce neural structures, while the more lateral parts which elaborate it in smaller quantities, would induce neural crest." Since the cells that will ultimately develop into dorsal neural tube are initially immediately adjacent to the nonneural ectodermal cells, these could provide a signal for dorsal differentiation similar to the notochord-derived *Shh* for ventralization of the neural tube. This idea has been postulated for a number of years in various forms but has only recently been tested with perturbations of specific candidate-inducing molecules.

Several lines of evidence now support the hypothesis that the ectoderm provides the molecular signals to promote dorsal differentiation in the lateral regions of the spinal cord, and likely in the more anterior regions of the neuraxis. Moury and Jacobson (1990) first tested whether interactions between the neural plate and the surrounding ectoderm were responsible for the induction of neural crest by transplanting a small piece of the neural plate from a pigmented animal to the ventral surface of the embryo. When the embryo was allowed to develop further, the transplant rolled into a small tube and at the margins gave rise to neural crest cells, as evidenced by the pigmented melanocytes that migrated from the ectopic neural tissue. These results were extended by the similar experiments of Selleck and Bronner-Fraser (1995) in the chick embryo, and in addition, they used an explant culture system, in which neural plate and epidermis were co-cultured and analyzed for proteins and genes normally expressed by neural crest. They found that the neural crest was induced to form from the neural tube when placed adjacent to the epidermis. The initial steps toward identifying the crest inducer were made by Liem et al. (1995). BMPs, discussed in the previous chapter for their role in neural induction, also play important functions in specifying dorsal regional identity in the developing spinal cord. Liem et al. (1995) used a similar explant culture system as that used for the analysis of *Shh* effects on ventralization of the neural tube. The neural tube was dissected into a ventral piece, a dorsal piece, and an intermediate piece (Figure 2.27). They then analyzed the expression of genes normally restricted to either the dorsal neural tube or the ventral neural tube to determine whether these genes were specifically induced by co-culture with the ectoderm. They found that certain dorsally localized genes, such as *pax3* and *msx1*, are initially expressed throughout the neural tube and are progressively restricted from the ventral neural tube by *Shh* from the notochord and floorplate. However, co-culture with the ectoderm was necessary to induce the expression of other, more definitive, dorsal markers, such as *HNK1* and *slug*. BMPs were found to effectively replace the ectodermally derived signal,

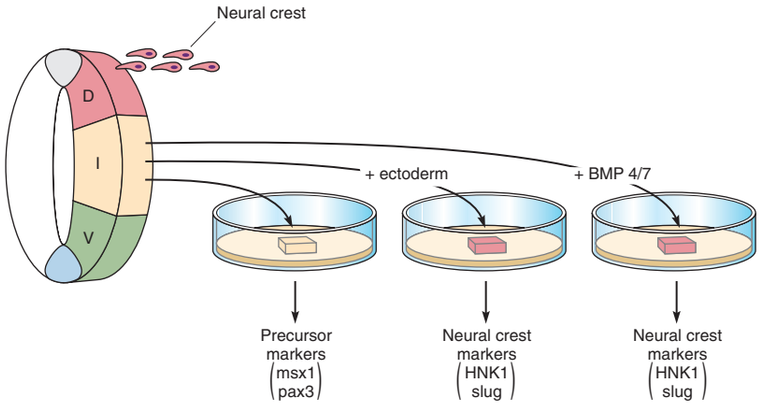

FIGURE 2.27 Liem et al. (1995) used an explant culture system to define the signals that specify dorsal cell fates. The neural tube was dissected into a ventral piece, a dorsal piece, and an intermediate piece, and the expression of genes normally restricted to either the dorsal neural tube or the ventral neural tube was used to determine whether these genes were specifically induced by co-culture with the ectoderm. They found that certain dorsally localized genes, like *pax3* and *msx1*, are initially expressed throughout the neural tube and are progressively restricted from the ventral neural tube by *Shh* from the notochord and floorplate; however, co-culture with the ectoderm was necessary to induce the expression of other, more definitive, dorsal markers, like *HNK1* and *slug*. BMPs were found to effectively replace the ectodermally derived signal, since these could also activate *HNK1* and *slug*, even from ventral explants.

since these could also activate *HNK1* and *slug*, even from ventral explants. Thus, there appears to be an antagonism between *Shh* from the ventral neural tube and BMPs from the dorsal neural tube; when BMP is added along with *Shh* to the explants, the *Shh*-induced motoneuron differentiation is suppressed.

In addition to the BMP signal that defines the border of the neural tube, there is evidence that the *wnt* signaling pathway plays a critical function in the specification of the neural crest fate (Deardorff et al., 2001). Treatment of neural plate explants with *wnt*, like those described for BMPs, is also sufficient to induce neural crest markers in the cells (Garcia-Castro et al., 2002), while blocking *wnt* signaling perturbs neural crest development. Several *wnt* genes are expressed in the developing ectoderm, adjacent to the point of origin of the crest, including *wnt8* and *wnt6*. Using a transgenic zebrafish line with a heat-inducible inhibitor of *wnt* signaling, Lewis et al. (2004) were able to precisely define the time in development when cells require the signal to become crest. They found a critical period when inhibiting *wnt* signaling was able to prevent neural crest development without affecting development of neurons in the spinal cord.

The model of dorsal-ventral polarity in the spinal cord that has emerged from these studies is as follows: BMPs and *wnt*s, expressed at the margin of the neural plate, induce the development of neural crest at the boundary of the neural plate and the ectoderm (Figure 2.28). BMPs and *wnt*s are also important for the development of the dorsal fates within the neural tube. *Shh*, expressed first in the notochord and later in the floorplate, induces ventral differentiation in the neural tube. The *Shh* and BMP/*wnt* signals antagonize one another, and through this mutual antagonism they set up opposing gradients that control both the polarity of spinal cord differentiation and the amount of spinal cord tissue that differentiates into dorsal, ventral, and intermediate cell fates. Much more will be said about the later stages of development of spinal cord cells in Chapter 4.

PATTERNING THE CEREBRAL CORTEX

The cerebral cortex, the largest region of the human brain by far, is not a homogeneous structure, but rather has many distinct regions, each of which has a dedicated function. It has been known for over one hundred years that there are significant variations in the cellular structure (cytoarchitecture) of the cortex from region to region. The different regions of the cerebral cortex were exhaustively classified into approximately 50 distinct areas by Brodmann (1909). Although all neocortical areas have six layers, the relative number of cells in each layer and the size of the cells are quite variable and specialized to the specific function of that area. For example, the visual cortex, a primary sensory area, has many cells in layer IV, the input layer, whereas the motor cortex has very large neurons in layer V, the output layer.

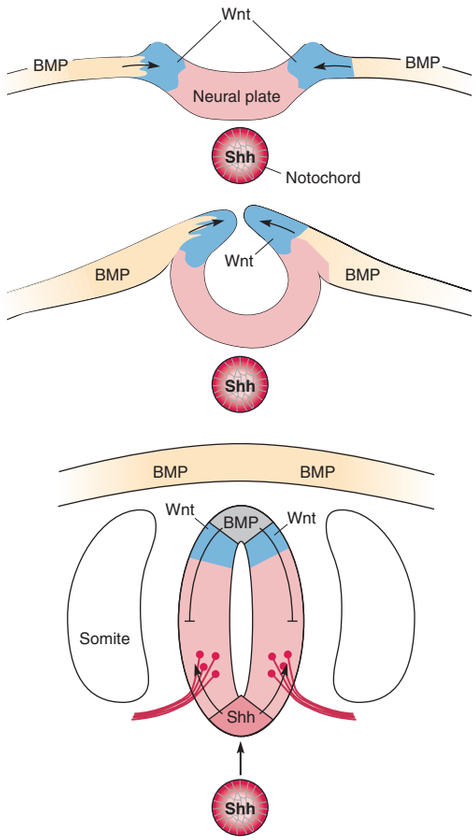

FIGURE 2.28 *Shh* is expressed first in the notochord and later in the floorplate and induces ventral differentiation in the neural tube. BMPs are expressed in the ectoderm overlying the neural tube and then in the dorsal neural tube cells later in development. These two signals antagonize one another, and through this mutual antagonism they set up opposing gradients that control both the polarity of spinal cord differentiation and the amount of spinal cord tissue that differentiates into dorsal, ventral, and intermediate cell fates.

Although for many years it has been thought that these different specializations occur later in development, as a consequence of the specific connections with other brain regions, more recent data indicates that the different areas have distinct identities much earlier in development, and these identities are not altered by changes in innervation (see Grove and Fukuchi-Shimogori, 2003). Like the other brain regions we have been discussing, the cerebral cortex arises from a layer of progenitors that comprises the early neural tube. In the specific case of the cerebral cortex, the anterior-most part of the neural tube, the telencephalon, is the source of these progenitors (Figure 2.1). The regional identities of the cortical areas can be monitored through the analysis of transcription factor expression. Two transcription factors that appear to have a role in the specification of regional identities in cortex are *pax6* (which we have already encountered for its role in eye development) and *emx2*. (Bishop et al., 2000; Mallamaci et al., 2000; Muzio et al., 2002) These two genes are expressed in opposing gradients across the cortical surface (Figure 2.29). *Emx2* is expressed most highly in the caudo-medial pole, while *pax6* is expressed highest at the rostral-lateral pole. Mutations in *pax6* cause an expansion of *emx2*'s domain of expression and ultimately an expansion of the areas normally derived from the caudal medial cortex, such as the visual cortex. Mutations in *emx2,* by contrast, cause the *pax6*-expressing domain to expand, and ultimately result in an expansion of the frontal and motor cortical regions.

The graded patterns of expression of *emx2* and *pax6*, along with the many examples of signaling centers we have already encountered in other regions of the developing nervous system, have led many investiga-

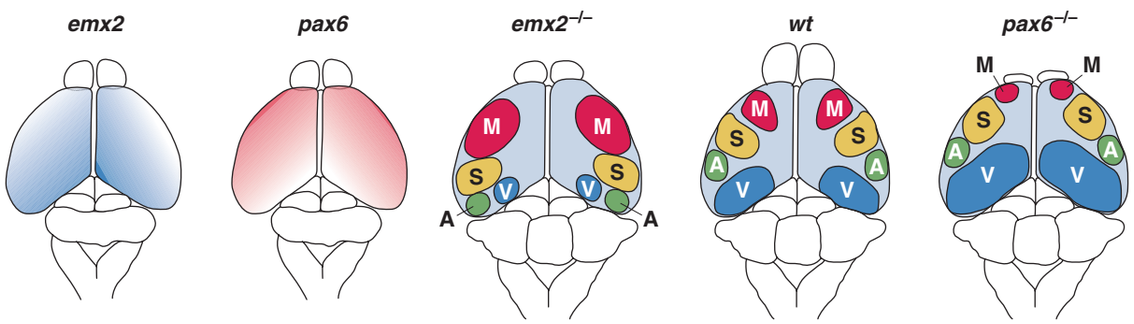

FIGURE 2.29 Two transcription factors critical for the specification of regional identities in the cortex are *pax6* and *emx2*. *Emx2* is expressed primarily in the posterior cerebral cortex and then gradually diminishes in expression toward the rostral cortical pole; *pax6* has the complementary pattern of expression. Loss of either the *pax6* gene or the *emx2* gene affects the cerebral cortical pattern of development. In the wild-type (wt) animal, the motor cortex (M) is primarily located in the rostral cortex, and the other sensory areas for somatosensation (S), auditory sensation (A), and visual perception (V) are located in the middle and posterior cortex, respectively. In the *emx2*-deficient mice, the pattern is shifted caudally, and a greater area is occupied by the motor cortex; by contrast, in the *pax6*-deficient mice, the visual cortex is expanded and the motor cortex is severely reduced. (Modified from Muzio and Mallamaci, 2003)

tors to postulate that similar signaling centers adjacent to the cortex regulate the regional expression of these transcription factors. We have already encountered the two most well-studied cortical patterning signals, FGF and retinoic acid. The most dramatic results have come from the studies of *fgf8*. *fgf8*, along with related *FGFs*, *fgf17*, and *fgf18*, are all expressed at the anterior pole of the developing telencephalon. To analyze the role of the *FGF*s in specifying cortical areal identity, Grove and her colleagues have misexpressed *fgf8* in different positions within the developing cortex (Grove and Fukuchi-Shimogori, 2003). These studies have monitored the identity of cortical regions both using the expression of region-specific transcription factors, like *pax6* and *emx2*, as well as analyzing later-developed properties of a region, like the barrel fields of the somatosensory map. Increasing the amount of *fgf8* in the anterior pole causes a downregulation of *emx2* and a caudal shift in the cortical regions, with an expansion of the rostral regions (Figure 2.30). Blocking the endogenous *fgf8* signal, by expressing a nonfunctional FGF receptor to bind up all the available *fgf8*, causes the opposite result, a rostral-wards shift in the cortical regional identities. Most dramatically, placing a source of *fgf8* in the caudal cortex causes the formation of a duplicated, mirror image of cortical regions.

The graded pattern of expression of *emx2* and *pax6*, in part regulated by *fgf8* and other *FGF*s from the anterior pole, appears to represent an early stage in the process by which areas of the cerebral cortex become specialized for different functions. As we saw for the segmentation of the fly embryo at the beginning of the chapter, patterning is often accomplished by an initial gradient of expression that becomes further subdi-

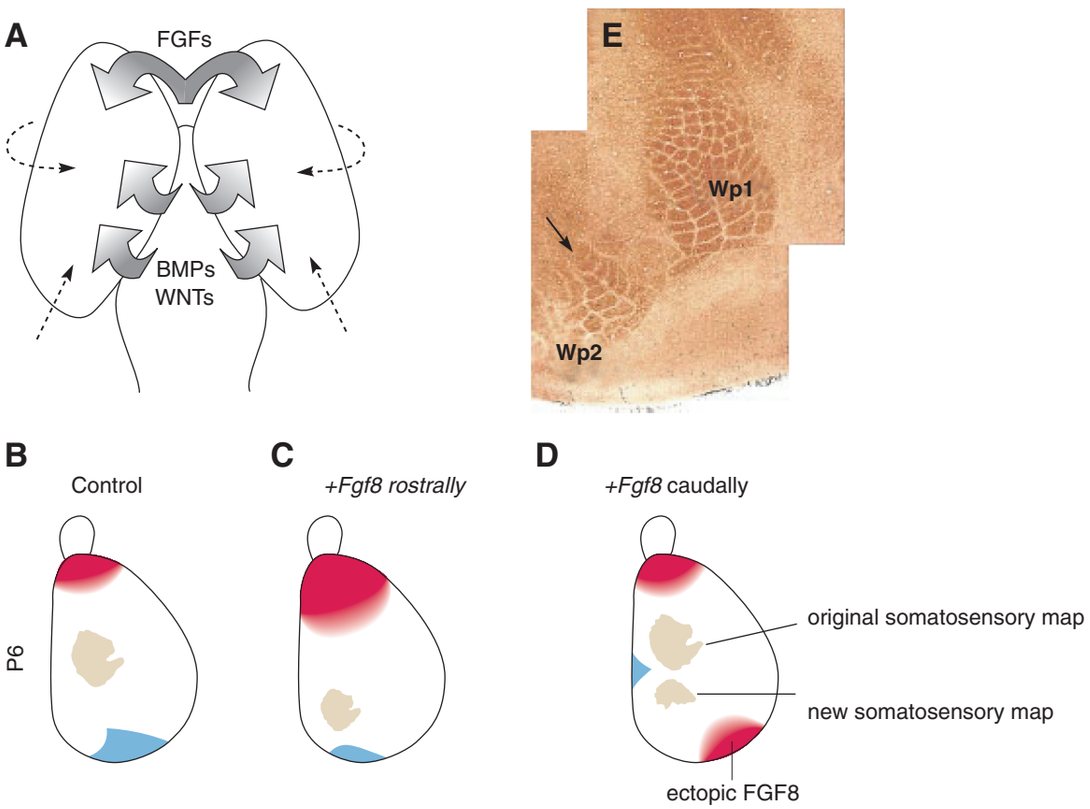

FIGURE 2.30 *Fgf8* patterns the cerebral cortex. A. *fgf8* is expressed at the anterior pole of the developing telencephalon, while BMPs and *wnt* genes are expressed in the posterior pole. Grove and her colleagues have misexpressed *fgf8* in different positions within the developing cortex. B. In the normal mouse, the barrel fields of the somatosensory map (yellow) are located near the middle of the cerebral cortex while *fgf8* (red) is expressed anteriorly and BMP (blue) is expressed posteriorly. C. Increasing the amount of *fgf8* (red) in the anterior pole causes a caudal shift in the cortical regions, including the somatosensory map. D. Placing a bead of *fgf8* in the caudal cortex causes the formation of a duplicated, mirror image of the somatosensory map. E. Micrograph of duplicated somatosory maps after the addition of an ectopic *fgf8* bead. Wp1 is the original map and Wp2 is the new map. (Modified from Grove and Fukuchi-Shimogori, 2003)

vided into finer and finer regions over time. The drive toward specialization seems to be fundamental to biology at all levels, from cells, tissues, organisms, and biological communities, and the cerebral cortex, arguably the basis for human preeminence, is no exception.

SUMMARY

The understanding of how the basic pattern of the nervous system is established has been put on a solid molecular ground in the past decade. One of the basic principles that has emerged from this work is that graded concentrations of antagonizing diffusible molecules are critically involved in setting up these patterns. These diffusible signaling molecules act to restrict the expression of specific transcription factors, which go on to regulate the expression of downstream target genes specific for the regional identity of part of the nervous system. One particularly well-conserved class of transcription factors, the *Hox* genes, is important in establishing and maintaining the regional identity of cells and tissues along the anterior-posterior axis of vertebrates throughout the hindbrain and likely the spinal cord. This conceptual framework holds true for vertebrates and invertebrates, and indeed, many of the molecular systems for generating specific parts of the nervous system have been highly conserved over the millions of years of evolution and considerable morphological diversity of animals.