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# Anteroposterior and Dorsoventral Patterning

Diana Karol Darnell

## PRINCIPLES AND MECHANISMS OF PATTERNING

If development is the process of reproducibly taking undifferentiated tissue and making it more complex in an organized way, then pattern formation is the mechanism for producing the organization in that complexity. This requires initiating differential gene expression within two or more apparently homogeneous cells. In some organisms this is initially done by segregating cytoplasmic determinants into specific daughter cells. These cytoplasmic determinants (proteins or RNAs) can result in the transcription of a restricted set of genes and begin the cascade that sets up tissues as different from one another in a coordinated pattern (Fig. 1). This is a totally cell autonomous mechanism and theoretically it could be the only mechanism for patterning the embryo. However, whereas this mechanism is well supported by evidence in the initiation of pattern formation in many invertebrates (e.g., *Drosophila*) and is probably invoked in vertebrates when asymmetrical cell division is the rule (e.g., stem cells), it does not appear to be the main method for embryonic pattern formation in vertebrates.

Vertebrate pattern formation, including the patterning of the nervous system, involves cellular responses to environmental asymmetries. Whereas embryonic cells initially may be a homogeneous population, they are not homogeneous in their relationship to asymmetrical environmental signals; by definition some are closer and some are further away. Thus, some receive a higher level of the signal and some a lower level or none at all. This difference gets translated into differential cellular response, which results in pattern formation within the field (Fig. 2).

Understanding pattern formation in the vertebrate nervous system means understanding this cascade of cellular and molecular interactions. The term **cascade** is often used to describe the events in development and pattern formation because one or more simple asymmetries initiate a pattern, which then becomes the foundation for the formation of a more complex pattern, which in turn forms the foundation for even finer patterning. The players in such a cascade are the cells and molecules of the early embryo. They include the *source* of the environmental asymmetry, which secretes the *signal*, which binds to the *receptors*, which initiate the *signal transduction pathway* within the responding cells, which activates the *transcription factors*, which regulate the set of coordinated *downstream genes* whose expression is modulated (up or down) as a result. These downstream genes may code for new signals, receptors, signal transduction proteins, transcription factors, or extracellular-, membrane bound-, cytoplasmic-, or nuclear-*facilitators* or -*antagonists* to modulate the system (Fig. 3), adding the next layer to the cascade.

The asymmetrical environmental cues often come from neighboring embryonic tissues whose early differentiation has made them into signaling centers. If these signaling centers can both induce *differentiation and pattern* in an undifferentiated field, they are called **organizers**, after the first such center to be identified, the Spemann–Mangold Organizer in amphibians, which was observed to induce and pattern the neuraxis (Spemann and Mangold, 1924). The signaling molecules may be peptide growth factors, vitamin metabolites, or other soluble, transported, or tethered ligands. When they have different effects across a homogeneous field of responding cells depending on their concentration, these signaling molecules are called **morphogens**. Because they invest the cells within the field with information about their relative position, they are also called **positional signals**. Models involving differences in binding affinity have been offered to demonstrate how one signal could have differing affects at different concentrations (Fig. 4).

Regardless of mechanism, these signals activate or induce the expression of a specific set of transcription factors that are unique to responsive cells at a particular distance from the source, and thus at a particular location in the embryo. These transcription factors are called **positional identity** genes, and they are often used as markers to define a region. Before the molecular revolution, they were called **positional information**. These transcription factors regulate the expression of selected genes, which may code for a component in this or another patterning pathway, or for proteins involved in differentiation of these cells. In the nervous system, this could include proteins mediating neuronal migration, axon outgrowth and navigation, precise connections, specific neurotransmitter production, or receptors that characterize the neurons of this locale. In the event that these downstream genes are unique to this region, they

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

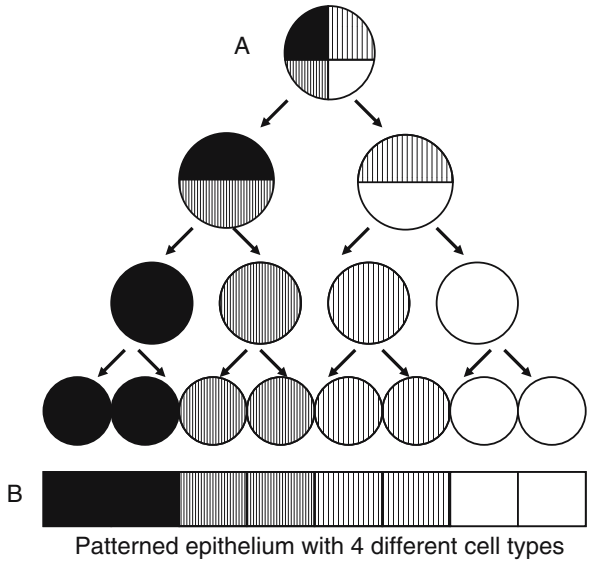


**FIGURE 1.** A patterned layer of cells can be achieved by localizing cytoplasmic determinants (shown here as various textures) within the parent cell. (A) Cell division segregates these determinants into different daughter cells, and they instruct their descendants (B) to acquire different phenotypes or fates. Cytoplasmic determinants are often RNAs for- or transcription factors themselves.

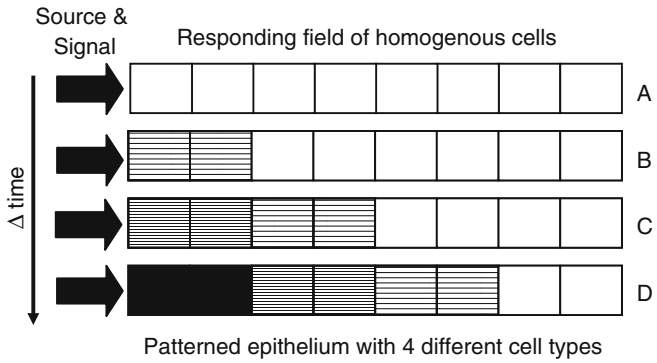


**FIGURE 2.** Asymmetric signaling (arrows) can change the fates of homogenous cells (white blocks) within the signal's reach. Cell fates can be specified in a stepwise pattern (as shown here, A > B > C > D) or all at once (A > D), depending on the timing of competence in the responding cells. This figure represents the formation of four different cell types (D) in response to a developing concentration gradient of a signaling molecule. Initially (A), the signal is low even near the source, but continued secretion yields a high concentration near the source and the possibility of inducing different cell types at several thresholds.

can also be used as **markers** when assessing the patterning or differentiation of the tissue.

The functions of various genes in these pathways are assessed through three types of experiments. First, candidate genes are identified because their expression shows a **correlation** with the timing and position of an observed patterning event. Second, the ectopic expression of the gene or presence of the protein causes a **gain of function**, showing that this gene product is **sufficient** to induce the observed pattern. Finally, failure to express the gene in the normal area results in a **loss of function**, indicating that the product is **necessary**. Evidence that a gene product is present, necessary, and sufficient is required to demonstrate a cause and effect relationship between the gene expression and the patterning event.

## Model Organisms

The current understanding of vertebrate neural pattern formation is due to research in a variety of model organisms including frog and other amphibians, chick, mouse, and zebrafish. Research with amphibians and birds has provided us with information on tissue interactions associated with patterning due to their accessibility to microsurgical manipulation, and more recently with specific localized protein function through

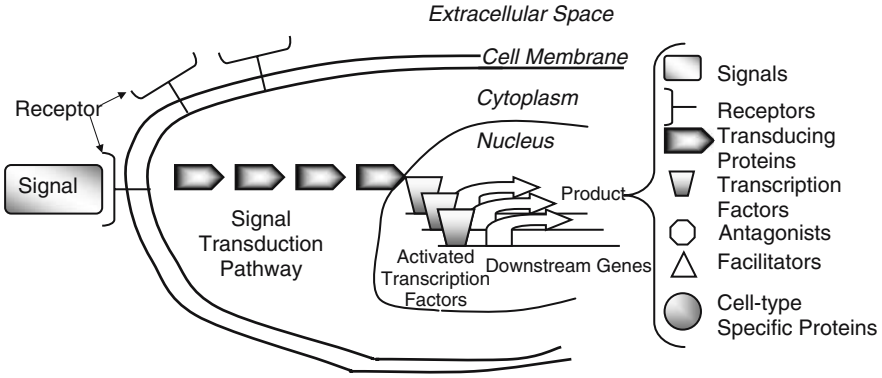


**FIGURE 3.** Pattern formation in vertebrates involves a signaling cascade that produces protein products, which can act in this cell or in the extracellular space to modify some aspect of a future signaling event. In addition, cell-type specific genes can be expressed leading to differentiation. Receptors may be membrane bound (as shown) for peptide ligands, or cytoplasmic as with RA and steroid ligands. Antagonists and facilitators can act in the extracellular space, in the membrane in conjunction with the receptor, with the signal transduction proteins or with a transcription factor. A transcription factor and its associated binding proteins can either up- or downregulate transcription of a given downstream gene.

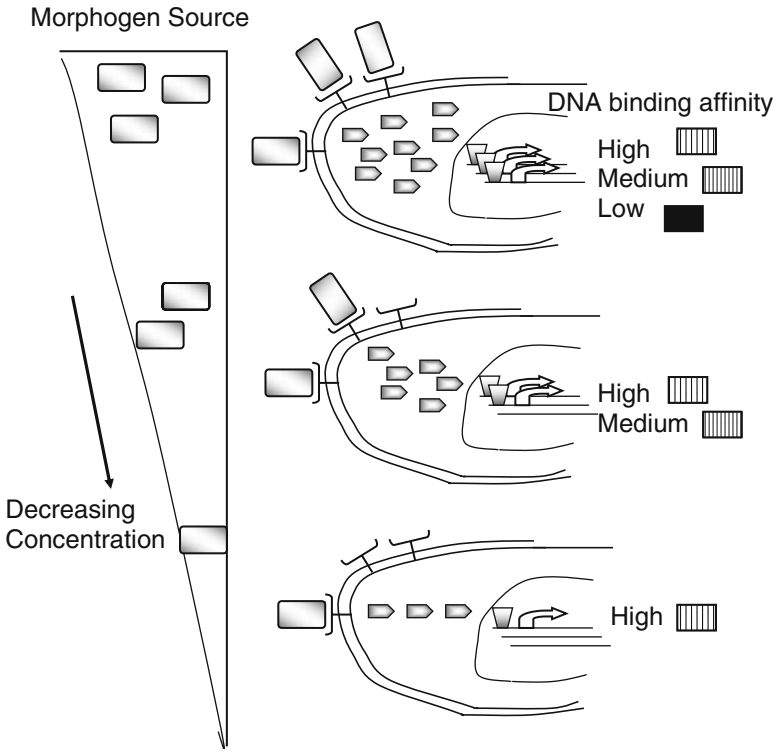


**FIGURE 4.** Model of morphogen action. Different concentrations of morphogen activate variable amounts of intracellular transcription factors. Downstream genes with variable affinity for these transcription factors are therefore activated at different concentrations of the morphogen. For example, at high levels of BMP (see Dorsal Patterning), high levels of nuclear SMAD activity would activate epidermal genes with low binding affinity (top cell), at intermediate levels neural crest genes would be activated (medium affinity, middle cell), and at low levels neural genes would be activated (high affinity, bottom cell). (Adapted from Wilson *et al*., 1997, with permission from the Company of Biologists Ltd.)

injection (frog) or transfection (chick) with corresponding genes or mRNA. Mouse has allowed us to eliminate (or add) specific genes, individually or in combination, to understand their importance in specific pathways. Zebrafish has been useful for its ease of mutation, which has helped identify new players and reveal their importance in the signaling pathways.

In many cases, the molecular pathways and cellular responses that have been identified appear to be conserved between all vertebrates. In fact, for some molecular pathways, the conservation reaches back to our common ancestors with insects; the same pathways are used in *Drosophila*. In others, there appear to be differences in pattern regulation that are specific to classes

of vertebrates. The best described of the general vertebrate central nervous system (CNS) patterning cascades include the anteroposterior (AP) patterning of the midbrain and hindbrain (reviewed by Lumsden and Krumlauf, 1996), and the dorsoventral (DV) patterning of the spinal cord (reviewed by Tanabe and Jessell, 1996; Lee and Jessell, 1999; Litingtung and Chiang, 2000). These will be discussed, and what is known about other regional CNS patterning pathways will be mentioned to highlight our current understanding of neural pattern formation.

## Axes of the Nervous System

The vertebrate nervous system is initially induced as an apparently homogeneous epithelial sheet of ectoderm adjacent to its organizer (see Chapter 1). This **neural plate** has contact ventrally with the underlying dorsal mesoderm, and laterally with the epidermal ectoderm, and these two neighboring tissues assist the neural plate to form a **neural tube** in a generally rostral to caudal sequence. Subsequently, a number of broad, discrete regions will form, both anteroposteriorly and dorsoventrally, beginning the cascade of specialization that will ultimately give rise to the complex vertebrate CNS (Fig. 5). Traditionally we identify the prominent AP regions as forebrain, midbrain, hindbrain, and spinal cord, whereas in the DV plane (at least in the trunk) we recognize the dorsal sensory neurons and ventral motor neurons. In addition, from the lateral margins of the early neuroectoderm, the sensory placodes and neural crest form and generate the cranial nerves and the peripheral nervous system (PNS; Fig. 5, see also Chapter 4). At later stages, left vs right also becomes an important feature of the differentiated nervous system; however, virtually nothing is known at this time about the control of this patterning. The cellular and molecular mechanisms associated with the AP and DV cascades of patterning that give rise to distinctive regional development in the early vertebrate neuroectoderm is the focus of this chapter.

# AP PATTERN

## Early Decisions

At its inception, the neural plate has three axes, AP, mediolateral, and left–right. As it forms the neural tube, the AP axis comes to extend virtually the entire length of the dorsal embryo. Patterning in the AP plane proceeds from coarse to fine subdivisions and involves morphogens, receptors, internal and external regulators, signal transducers, transcription factors, and tissue specific target genes. The embryo matures in a head to tail direction, so more anterior structures are further along in their developmental cascade than are caudal structures. Thus, it is often not entirely meaningful to state the subdivisions as though they have formed concurrently. The AP cascade is much more complex than that. However, for simplicity's sake we say that the early neural plate begins its life in an anterior state (defined here as "head"), and the first step in patterning is to establish from this a separate "trunk" region. Soon thereafter, beginning at the anterior end of the embryo, the neural plate forms a neural tube, which swells, extends, and further subdivides to form the **prosencephalon** or forebrain, the **mesencephalon** or midbrain, the **rhombencephalon** or hindbrain, and the narrow **spinal cord** (Fig. 6). Conventional embryology and anatomy include the forebrain, midbrain, and hindbrain with the head, and begin the trunk at the anterior spinal cord (either just caudal to the last rhombencephalic swelling at r7 and the first somite, or at the level of the fifth somite and first cervical vertebrae). However, evolutionarily, it appears that the hindbrain level of the AP axis may have come first in prevertebrate chordates, with structures anterior (new head) and posterior (trunk and tail) being added as vertebrates evolved. Within the realm of neural pattern formation, this "new head" including the forebrain and midbrain express Otx2 and other non-Hox transcription factors as positional information, and are dependent for their formation on several signaling factors called "head inducers" (see below), making this region of the head distinctly different from the hindbrain. In contrast, the spinal cord is clearly patterned as an extension of the hindbrain using *Hox* genes as positional information, and is dependent for its formation on several caudalizing factors, which are antagonistic to those involved in "new head" formation. Thus, for the purposes of discussing pattern formation, "head" will be defined as the neuroectoderm rostral to the midbrain/hindbrain boundary (site of the **isthmic organizer**), and "trunk" as the area caudal to it (including the future hindbrain and spinal cord). This "head–trunk" division represents a didactic effort to segregate major patterning differences.

Within the head and trunk further subdivisions are established in response to asymmetric signals through the expression of positional information genes (region specific transcription factors), and these regions in turn are also subdivided until the finely patterned detail of the fetal CNS is achieved. Details of our understanding of the pathways leading to these major and minor subdivisions appear below.

### First Division

The longstanding models for AP patterning are founded on landmark experiments from the early part of the last century (Spemann and H. Mangold, 1924; Spemann, 1931; O. Mangold, 1933) and reconsidered in the 1950s by Nieuwkoop (Nieuwkoop *et al*., 1952) and Saxen and Toivonen (reviewed by Saxen, 1989). Working with amphibian embryos, Spemann and H. Mangold discovered that the upper (dorsal) blastopore lip could induce a well-patterned ectopic neural axis. They called this region the organizer. Subsequently, Spemann (1931) determined that the organizer of younger embryos could induce a whole axis including head while older organizers could only induce the trunk neuraxis. Similarly, O. Mangold determined that the underlying mesendoderm having ingressed from the organizer at early stages induced the head, whereas the later mesoderm induced the trunk. Thus the concept of head and trunk as the first coarse AP division of the neuroectoderm was established.

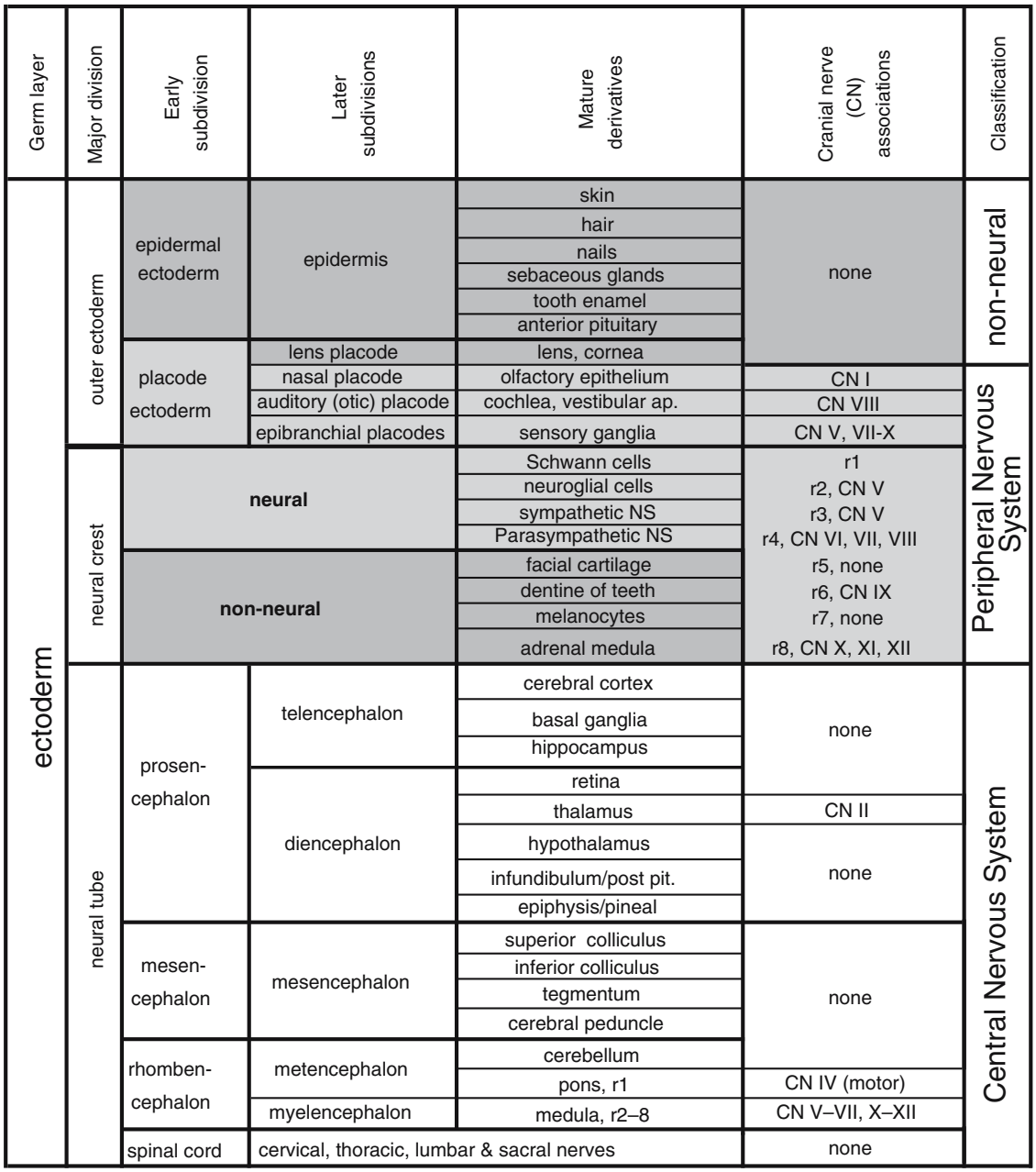


**FIGURE 5.** Chart showing developmental progression of ectodermal differentiation. CN, cranial nerves are: I Olfactory (special sensory), II Optic (special sensory), III Oculomotor (motor and autonomic), IV Trochlear (motor), V Trigeminal (sensory and motor), VI Abducens (motor), VII Facial (motor, sensory, and autonomic), VIII Auditory/Vestibulo-acoustic (special sensory), IX Glossopharyngeal (sensory, motor, and autonomic), X Vagus (autonomic, sensory, and motor), XI Accessory (motor and autonomic), XII Hypoglossal (motor). See also Fig. 12. Shading distinguishes major tissue classifications (CNS, PNS, Non-neural).

## Head Neural Induction and Maintenance

The major similarity between the early models of AP patterning is the understanding that the initial neuroectoderm induced is rostral in character, either by default or due to primary rostralizing signals that are present as the neural ectoderm forms. This understanding has been supported at the molecular level by observations that the neural inducers chordin, noggin, and follistatin (all Bone Morphogenic Protein or BMP inhibitors) are able to induce forebrain but not neuroectoderm of more posterior character in amphibian animal caps (see Chapter 1), whereas in mouse double mutants for chordin and noggin, the forebrain does not form (Bachiller *et al*., 2000). These experiments indicate these factors are both sufficient and necessary to form the head.

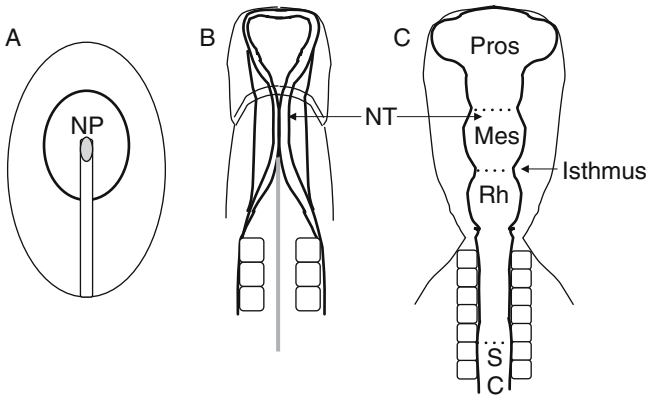


**FIGURE 6.** Drawings of avian embryos at various early stages. (A) At late stage 3, the neural plate (NP) (bold line) forms around the organizer (gray). (B) At stage 8, the neural plate rolls into a neural tube (NT) beginning at the future midbrain level. (C) At stage 11, the neural tube has formed its rostral vesicles, the prosencephalon (Pros) or forebrain, mesencephalon (Mes) or midbrain, and rhombencephalon (Rh) or hindbrain as well as the spinal cord (SC). Arrow shows the location of the isthmus, which forms an organizer between the mesencephalon and rostral rhombencephalon.

However, this one-step model of head formation appears to be an oversimplification because other proteins or tissues have been identified that are also sufficient and necessary for head formation. In mammals, there is a second signaling center, the anterior visceral endoderm (AVE) that secretes a TGFβ superfamily member (Nodal) and TGFβ and Wnt antagonist, Cerberus-like (cer1), that are involved in head formation. In many vertebrates cerberus and several other Wnt antagonists (Dickkopf-1 [Dkk1], Frzb1, and Crescent) are expressed in the rostral endoderm or cells in the early organizer, tissues which share head-forming qualities with the mammalian AVE. Ectopic expression of cerberus (in *Xenopus*; Cer, Bouwmeester *et al*., 1996) and Dkk1 (in *Xenopus* and zebrafish; Kazanskaya *et al*., 2000, Hashimoto *et al*., 2000) show these proteins are sufficient to produce anterior neural ectoderm from ectodermal precursors. In addition, *Xenopus* embryos posteriorized experimentally (with bFGF, BMP4, or Smads: See below) are rescued by Dkk1 (Hashimoto *et al*., 2000; Kazanskaya *et al*., 2000). Conversely, overexpression of head inducers in caudal neuroectoderm results in the loss of caudal markers and the expansion of more rostral fates. All of these experiments indicate that these "head inducers" are sufficient to support rostral neural formation. These proteins are probably also necessary, because injections of anti-Dkk1 antibody resulted in loss of the telencephalon and diencephalon, and null mutation of *Dkk* in mouse leads to loss of all head structures anterior to the hindbrain (Mukhopadhyay *et al*., 2001).

From these data we infer that these additional signaling factors induce head formation and this could be used to argue that anterior neuroectoderm is not the default state. On the other hand, rostral neural ectoderm could still be the default but undetermined state, and these factors could merely be required to protect it from transformation to more caudal fates in the presence of caudalizing signals. Because their function is the antagonism of Wnt action, and Wnts are caudalizing factors, it seems reasonable that anterior is the default and that "head inducers" like Cer and Dkk are required to override caudalizing factors to maintain (determine) the head in its original state (see below).

## Trunk Neural Induction

Whereas the early modelers of AP pattern agreed that head neuroectoderm was primary, they differed in their ideas of how more caudal neuroectoderm was formed (Fig. 7). The Spemann/Mangold model proposes that the cells in the early organizer induce and pattern the head, whereas at a later stage these cells are replaced with a population that induces the trunk neuroectoderm. Thus the organizer shifts from inducing the head to inducing the trunk over time (temporal separation) through the movement of cells (spatial separation). Nieuwkoop and coworkers proposed that signals (called transformers) from some other source could convert some of the rostral neuroectoderm into caudal neuroectoderm. Saxen and Toivonen proposed opposing gradients of morphogens whose relative levels would establish appropriate AP patterning separate from neural induction. One major difference between the models is whether a neural inducing and caudalizing signal is relayed through the organizer and coupled to induction or whether a caudalizing signal from a nonorganizer source transforms already-induced neuroectoderm directly by acting in a competitive or antagonistic manner. In the end, there is no reason that all of these pathways could not be used during AP patterning of the nervous system, and indeed, evidence indicates that they are (Kiecker and Niehrs, 2003).

Evidence in support of the Spemann/Mangold head- and trunk-organizer (Fig. 7A) model comes from several sources. First, classic amphibian and avian grafting experiments show that young organizers can induce a complete axis, whereas older organizers have lost the ability to induce the head. Second, "Keller sandwich" experiments, in which the amphibian neural ectoderm extends without underlying mesoderm, show that AP neural patterning can result from planar signals from the organizer (reviewed by Doniach, 1993; Ruiz i Altaba, 1993, 1994).

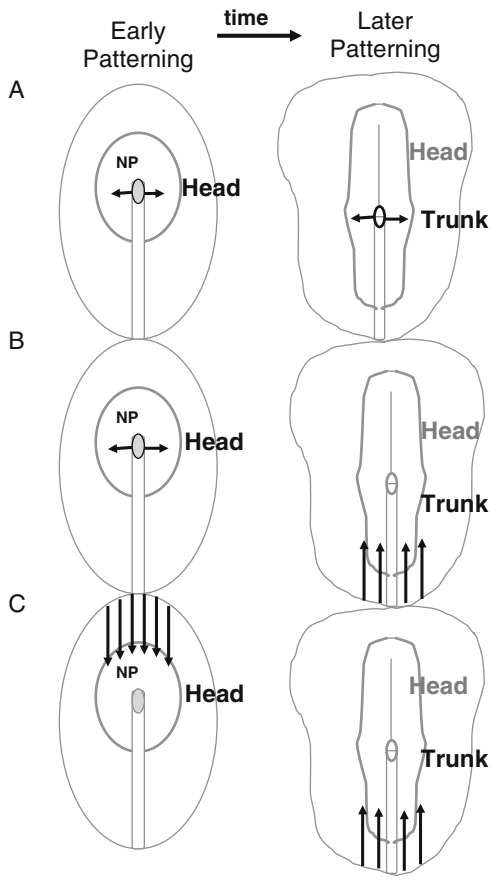


**FIGURE 7.** Three models of initial neural pattern formation. Arrows indicate patterning signals. (A) The Spemann/Mangold model wherein early signals from the organizer pattern the head and later signals from the organizer pattern the trunk. (B) The Nieuwkoop model wherein early signals from the organizer pattern the head and then later signals from other sources transform more caudal neuroectoderm into trunk. (C) The Saxen & Toivonen model wherein a rostral gradient of anteriorizing signals patterns the head and a caudal gradient of posteriorizing signals patterns the trunk.

Third, if the trunk organizer is going to exist with separate function from the head organizer, then one needs evidence that the organizer changes its secretory molecules over time and that the later ones can cause caudalization of the neuroectoderm. This has been demonstrated in mouse where retinoic acid (RA), a caudalizing agent, is produced by the older node but not the younger (Hogan *et al*., 1992) and in *Xenopus*, where derivatives from the young node secrete chordin, which induces the head, whereas derivatives of older nodes secrete fibroblast growth factor (FGF), which induces the trunk (Tiara *et al*., 1997). In addition, older chick nodes can induce *Xenopus* animal caps to express Pax3, a caudal marker, whereas younger nodes cannot (Bang *et al*., 1997). Fourth, if the trunk organizer is going to be both inducing and patterning the trunk neuroectoderm in a single step, then a molecule that can both induce and caudalize must be identified. FGF is able to do both (Lamb and Harland, 1995). Fifth, there is evidence that trunk neuroectoderm is created *de novo* from later node and this generation requires FGF (Mathis *et al*., 2001). Finally, recent experiments have implicated BMP-4 as a signal that acts directly on the *Xenopus* organizer to convert it from a head inducer to a trunk inducer (Sedohara *et al*., 2002). Thus tissue interactions appropriate for the Spemann/Mangold model of AP pattern play a role in AP neural patterning.

Significant evidence also exists in support of the Nieuwkoop model (Fig. 7B). This model is usually called activation/transformation for the initial activation (induction and patterning) of the head neuroectoderm by the organizer, followed by the subsequent transformation of the caudal cells in this head field into trunk neuroectoderm. Classic amphibian experiments demonstrate that vertical signaling from the mesoderm can directly pattern the neuroectoderm induced by the organizer (reviewed by Doniach, 1993; Ruiz i Altaba, 1993). Several secreted factors capable of caudalization have been identified including FGFs, RA, and vertebrate homologs of the *Drosophila* wingless protein (Wnts). FGFs (in *Xenopus*) are expressed in the posterior dorsal mesoderm during gastrulation. When anteriorized animal caps (which form anterior neural ectoderm expressing Otx-2 (forebrain and midbrain) and En2 midbrain–hindbrain boundary) were treated with bFGF both anterior and posterior markers (Krox-20/hindbrain and Hoxb-9/spinal cord) were expressed. When a later stage of the neural ectoderm was treated with bFGF it induced forebrain to express a hindbrain marker

and hindbrain to express the spinal cord marker (Cox and Hemmati-Brivanlou, 1995). In another lab, Kengaku and Okamoto (1995) determined that progressively more posterior markers were induced when increasing concentrations of FGF were provided to neural ectoderm. Finally, recent work in zebrafish indicates that FGF3, through chordin (a BMP inhibitor), mediates expansion of the posterior- and suppression of the anterior neuroectoderm (Koshida *et al*., 2002). Thus, FGFs would fit the role of Nieuwkoop's transforming signal. But they are not alone.

Retinoids can also serve this function. Retinoids are expressed at high levels in the posterior neuroectoderm and are involved in establishing the positional information for the hindbrain. RA and other retinoid derivatives of vitamin A act as signaling molecules much as steroid hormones do. They are able to pass through the plasma membrane of cells and bind to retinoic acid receptors called RARs and RXRs (retinoid X receptor peptides) in the cytoplasm. These translocate to the nucleus and act as transcription factors by binding to retinoic acid response elements (RAREs) within the promoters of certain genes. *Hox* genes contain RAREs and their expression is modified by levels of retinoids acting as morphogens. That is, *Hox* genes with rostral expression patterns (e.g., in the rostral hindbrain) are expressed at low levels of retinoids, while more caudal *Hox* genes are expressed only where the levels of retinoids are higher. Blocking RA signaling results in the loss of caudal rhombencephalic pattern and the transformation of this region into more rostral rhombencephalon (Dupe and Lumsden, 2001; see Hindbrain Patterning below). Artificially raising the concentration of RA in the environment results in changes in the expression patterns of some regionally expressed transcription factors including *Hox* genes, demonstrating the relationship between this morphogen and these positional information transcription factors. Phenotypically, increased RA results in a loss of anterior structures and markers (Fig. 8A). Distinct phenotypes are generated depending on the timing of exposure to RA (in mouse) indicating that RA can influence differentiation at several steps in the AP axis cascade (Fig. 8B; Simeone *et al*., 1995).

Finally, a strong case can be made for Wnts as transformers in the caudalizing of the neuroectoderm. Overexpression of various Wnts, or of the elements in their canonical signal transduction pathway, or of lithium chloride, the artificial activator of this pathway, leads to loss of head structures and induction of posterior neural markers. Blocking Wnt activity leads to head gene expression, while mutations in various genes in this pathway lead to caudal truncations. Recently, Kiecker and Niehrs (2001) have shown that neuroectoderm associated with increasing concentrations of Wnt8 expresses genes associated with increasingly caudal levels of the neuraxis, demonstrating that Wnt, too, is a caudalizing morphogen. Thus, these three caudalizing morphogens, FGFs, RA, and Wnts, support the Nieuwkoop model of Activation and Transformation. By regulating the expression of positional identity genes within the already-formed anterior neuroectoderm, transforming signals can mediate posterior neural patterning.

Finally, the Saxen and Toivonen model (see Fig. 7C) seems to best express how the head is maintained in light of these transforming/caudalizing factors. But rather than a competition between two positive signaling gradients as originally proposed, we find the mechanism of head and trunk formation ultimately depends on antagonism gradients of inhibitors, comparable to the amphibian model for the induction of the neuroectoderm (Chapter 1; Fig. 9). In both cases, the default state is singular. In "neural induction" the default state of the ectoderm is neural (expressing transcription factors Sox1, 2, and 3). In "head induction" the default state is anterior ectoderm or head (expressing transcription factors Lim1, Otx2, and Anf ). To increase complexity during development, secreted signals appear with the ability to transform this uniform tissue into another. For neural induction they are BMPs, and the secondary state is epidermal ectoderm. For AP neural pattern, these signals include RA, FGFs, Wnts, and BMPs (Glinka *et al*., 1997; Piccolo *et al*., 1999) and the secondary state is more caudal neuroectoderm. In order to protect the first state from this modification, antagonists of these signal(s) are generated. In neural induction, these are noggin, follistatin, and chordin expressed in the organizer and its derivatives. For AP patterning, these could be proteins such as cerberus, dickkopf, nodal, and lefty (reviewed by Perea-Gomez *et al*., 2001), frzb, noggin, and crescent, which are secreted from the rostral mesendoderm and which are antagonists of Wnts, BMPs, and other signaling molecules involved in caudal specification. Successful protection of a subset of the original ectodermal region results in the formation of two separate potentials in each case (neural vs epidermal and "head" vs "trunk"). In addition, because the BMPs and caudalizers are morphogens, additional intermediate states can also be induced at the interface between these two states resulting in additional complexity. For neural induction, this begins the DV patterning cascade by inducing the neural crest, whereas for AP patterning the midbrain–hindbrain boundary or isthmus, appears to be the intermediate state. Thus, a three-step model of early AP pattern formation is supported: *Neural induction* (with anterior character), *caudalization* (new neural induction and transformation to generate trunk character), and *anterior maintenance* to protect two separate states, "head" and "trunk."

Although this three-step model is presented as a synthesis of the historical models that fits the current data, there are other ways of interpreting these data. One alternate interpretation still holds head induction to be the direct result of BMP and Wnt antagonism (an unmodified Saxen–Toivonen double-inhibitor model). This is supported by ectopic head induction using appropriate antagonists in *Xenopus* embryos (e.g., see Niehrs *et al*., 2001). These antagonists are sufficient for head induction, but because they are also required for head maintenance and the neural state may be the default, it is difficult to demonstrate whether they are or are not actually required for induction of the head.

In addition, there may be some important differences between model animals in the caudalizer-antagonism step of this AP patterning. Specifically, the required source of the secreted caudalizing-factor antagonists ("head inducers") in mammals is the AVE (reviewed by Beddington and Robertson, 1998), although grafts to other species indicate the mouse node/organizer also produces the appropriate signals to induce and

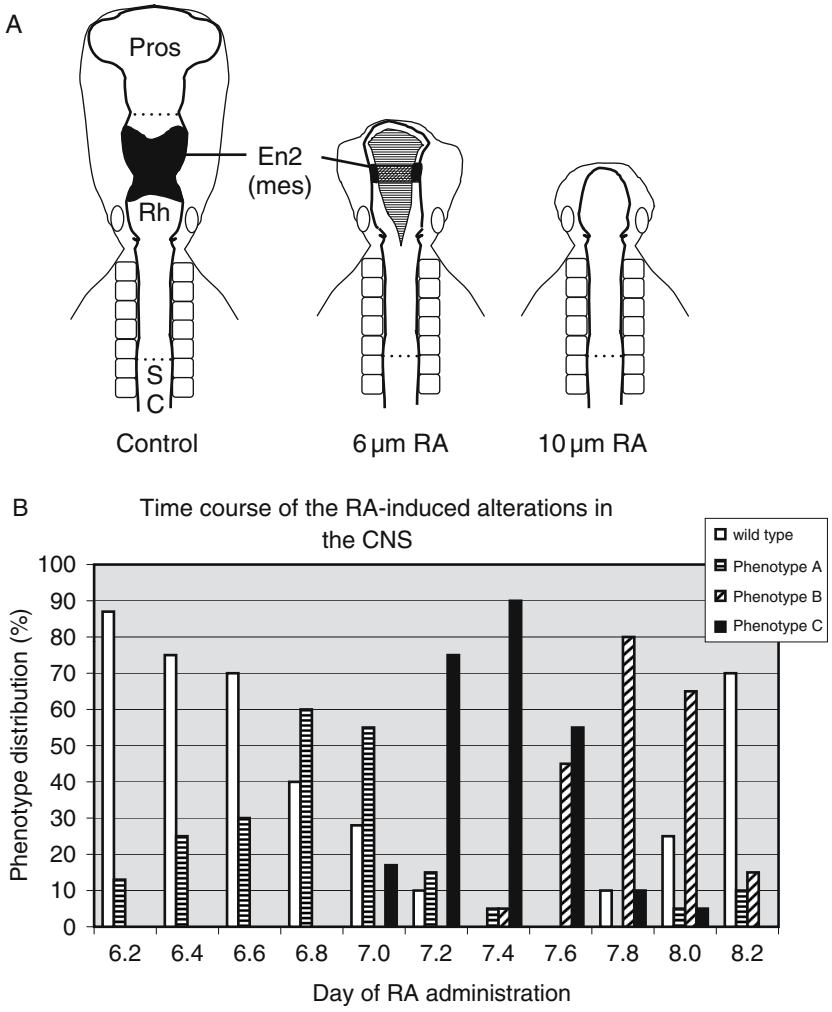


**FIGURE 8.** Effects of RA addition to developing CNS. (A) Diagrammatic representation of chick embryos treated with RA at stage 3 and cultured for 24 hr. Control embryos develop normal features and express En2 at the isthmus (solid black). Embryos treated with 6 μm RA express En2 in a smaller area and at lower levels. Embryos treated with 10 μm RA failed to express En2 or expressed it at levels undetectable with whole mount immunocytochemistry. Development of tissues rostral to the mesencephalon was not observed (Darnell, 1992). (B) 250–400 mouse embryos were analyzed for each time point and the percentage of each phenotype is shown on the graph. The wild-type phenotype dominates for RA treatment at both ends of the trial period, delineating the critical period for RA effect overall. The shifts in distribution between the other phenotypes indicates RA has different functions at different times during development. Phenotype A (mild: reduction in the olfactory pit and midbrain DV compression) reveals the structures most sensitive at 6.8 and 7 dpc. Phenotype B (severe, atelencephalic microcephaly: growth retardation; reduction or lack of anterior sense organs and neural vesicles back to the isthmus; branchial arches reduced or abolished and hindbrain disordered). Sensitive period 7.6–8.0 dpc. Phenotype C (moderate, anencephaly: hypertrophic obliteration of the ventricles, open neural roof for diencephalon through hindbrain, all anterior genes expressed but domains altered, for example, Hoxb1 expression expanded from normal r4, into presumptive r2–r3 territory). Sensitive period 7.2–7.6 dpc. (Redrawn after Simeone *et al*., 1995, Fig. 1.)

maintain head (e.g., see Knoetgen *et al*., 2000). Traditionally, in birds, fish, and amphibians the source of "head" inducers has been attributed solely to the early organizer/node and its derived prechordal plate mesendoderm, although this has been recently contested. In chick, the hypoblast, a tissue similar to the AVE, can transiently induce early head neural markers (Foley *et al*., 2000) and the foregut endoderm is involved in forebrain patterning (Withington *et al*., 2001). In fish, rostral endodermal cells are involved in anterior neural patterning through Wnt antagonism (Houart *et al*., 1998, 2002). And in *Xenopus*, endodermal expression of *Hex* (an AVE associated gene in mouse) is also involved in anterior patterning of the neuroectoderm (Jones *et al*., 1999). Thus, it now seems less likely that the two-source localization of early head maintainers in mammals is due to mutations that occurred in the signals localizing the expression of these genes after mammals diverged from other vertebrates. Instead, it may be a more primitive pattern that has been maintained more robustly or localized differently in small embryos where the

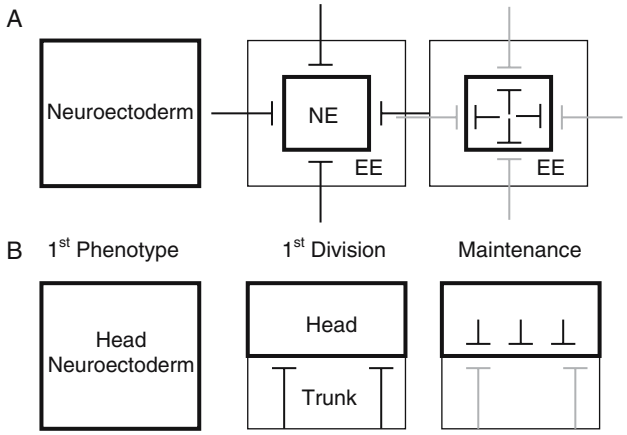


**FIGURE 9.** A comparison of the models for neuroectoderm "induction" and patterning. (A) The first phenotype of ectoderm is neuroectoderm. The first division of this tissue into two types occurs when inhibitory signals from the periphery (BMP) inhibit the neural signaling pathway and turn the outer area into epidermal ectoderm. The neural ectoderm is protected from these inhibitors by inhibitors from the organizer. (B) The first phenotype in patterning is head neuroectoderm. The first division of this tissue into two types occurs when signals from the caudal embryo transform closer neuroectoderm into trunk neuroectoderm. (These signals may either activate and/or inhibit certain gene expression.) The head is protected from these transforming signals by inhibitors expressed rostrally.

caudalizing signals would otherwise swamp out the rostral region. Experiments in diverse vertebrates with embryos of various sizes will be required to test this hypothesis.

## Regional Patterning

### Forebrain

The "head" is thus defined for pattern formation purposes as a region of anterior neuroectoderm that initially expresses the transcription factor Otx2 and extends from the anterior neural ridge at the rostral end of the embryo to the isthmus at the posterior margin of the future midbrain. Mouse mutants lacking Otx2 fail to form head structures (Acampora *et al*., 2001), whereas in *Xenopus*, Otx2 is sufficient to induce anterior neural genes (Gammill and Sive, 2001). Thus, this transcription factor provides positional information for the head.

This Otx2 field subsequently subdivides within in the AP plane to generate the more complex pattern associated with the later forebrain and midbrain. These subdivisions result from responses to patterning signals from the underlying mesendoderm or prechordal plate and from new sources of environmental asymmetry, the anterior neural ridge in the anterior head and the isthmus in the posterior head. These signals could induce the appearance of active, region-specific transcription factors that could subdivide and further pattern the head. For example, Otx2 spans the head at the neural plate stage. Later, Otx1 is upregulated in all but the rostral region of Otx2-expression, then Emx2 is upregulated in the middle of the Otx2 region and Emx1 in the middle of this. The Otx2 pattern is followed by neural tube closure and the formation of anatomically identifiable pattern within the neural tube (36 hr in chick, 8–9.5 days in mouse, 4 weeks in human) correlated with the expression of these later genes (Fig. 10; Boncinelli *et al*., 1993; Bell *et al*., 2001).

Anatomically the prosencephalon (forebrain) forms the telencephalon (rostral forebrain) and diencephalon (caudal forebrain). The telencephalon, which ultimately forms the cerebral isocortex, olfactory cortex and bulbs, hippocampus, and basal ganglia (striatum and pallidum) expresses all of the head transcription factors mentioned previously, plus BF1. BF1 is upregulated in the telencephalon and retina by FGF8 (Shimamura and Rubenstein, 1997), a signaling molecule that is expressed in the anterior neural ridge and at the isthmus. Because the mesencephalic neuroectoderm does not upregulate BF1 in response to FGF8 (rather it upregulates the isthmic gene *En2*), it is clear that differential competence is established regionally within the head prior to the expression of these later marker genes.

The patterning of the diencephalon (in chick) has been described (Larsen *et al*., 2001) but the signaling events required for this pattern formation have not been determined. The early diencephalon is subdivided into two functionally distinct regions: the anterior parencephalon and the posterior synencephalon. There is no cellular boundary (lineage or cell-mixing restriction) between the parencephalon and the telencephalon anterior to it; however, such a boundary does exist between the parencephalon and synencephalon (lineage restriction), and between the synencephalon and mesencephalon (lineage and cell-mixing restriction). Subsequently, the parencephalon is subdivided into ventral and dorsal thalamus by an anatomical feature called the zona limitans intrathalamica (zli), which is correlated with cells on either side becoming restricted to their compartment and with Gbx2 expression dorsally and Dlx2 and Pax6 expression ventrally.

Specific regulation of a number of other transcription factors has been correlated with the development of specific regions within the rostral head. For example, four POU-III transcription factor genes, *Brn-1*, *Brn-2*, *Brn-4*, and *Tst-1*, are expressed in the rat forebrain beginning on embryonic day 10 in a spatially and temporally complex pattern. The most restricted

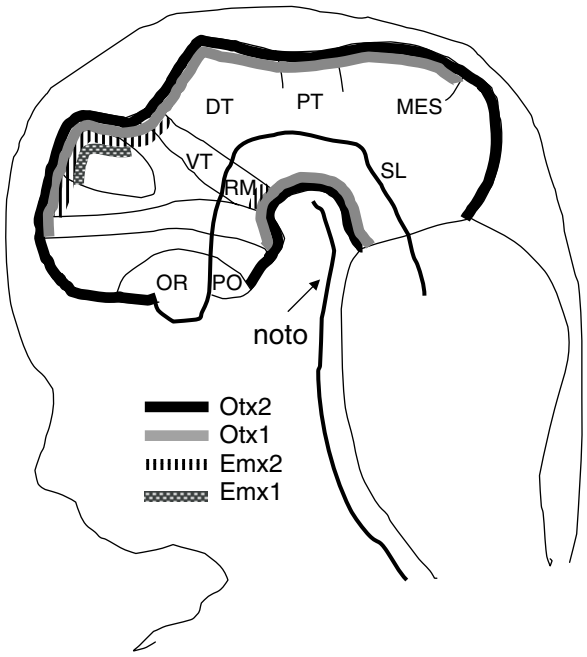


**FIGURE 10.** A diagram of the strong expression domains of four "head" genes in the mouse (E10). Internal lines correspond to locations where expression patterns change, indicating a possible functional boundary in AP patterning. Various anatomical subdivisions or precursor regions are labeled, including DT, dorsal thalamus; MES, mesencephalon; noto, notochord; OR, optic region; PO, post-optic; PT, pretectum; RM, retro-mammilary area; SL, sulcus limitans; and VT, ventral thalamus. (Redrawn after Boncinelli *et al*., 1993.)

of these is *Brn-4*, which is expressed in the striatum of the telencephalon and parts of the thalamus and hypothalamus within the diencephalon (Alvarez-Bolado *et al*., 1995). *Dlx*- and *Nkx2* gene families are regionally expressed in the diencephalon and other regions of the forebrain and their expression boundaries correlate with certain morphological boundaries (e.g., between isocortex and striatum within the telencephalon; Price, 1993). No clear boundaries of gene expression or cell-mixing restriction have been detected to subdivide the diencephalon into more restricted neuromeres, although the boundary between the diencephalon and mesencephalon is so defined (Larsen *et al*., 2001).

## Midbrain and Isthmus

Just caudal to the diencephalon, there is a bulge in the neural tube called the mesencephalon or midbrain. It is limited at its posterior margin by a constriction called the isthmus (see Fig. 6). The dorsal mesencephalon contributes to the superior and inferior colliculi (in mammals; equivalent to the optic tectum and torus semicircularis of birds), whereas the ventral mesencephalon (also known as tegmentum) generates structures such as the substantia nigra and the oculomotor nucleus. Otx2 is expressed broadly anterior to the isthmus, while the signaling molecule Wnt1 is expressed in a narrow band at the constriction. On the other side of the constriction, the transcription factors Pax2 and Gbx2 and signaling-molecule FGF8 are upregulated at the right time to be involved with the patterning of this region. Otx2 and Gbx2 appear to act as transcriptional repressors, each repressing the transcription of the other to generate a tight boundary of gene expression at the isthmus, which is required for the appropriate expression of Fgf8, Pax2, and En2 (Glavic *et al*., 2002). This boundary is not, however, a compartment boundary that limits cell movement across it (Jungbluth *et al*., 2001). Another transcription factor, Xiro1, is expressed in a domain that overlaps the expression of Otx2, Gbx2, and FGF8 and is required for their correct spatial regulation (Glavic *et al*., 2002).

Mouse mutants demonstrate that the signaling molecule Wnt1 and transcription factors En1/En2 expressed around this region are necessary for its development. Simultaneous knockouts of *En1* and *En2* result in failure of midbrain and cerebellar development. Knockouts of *Wnt1* show early expression of En1 and En2 but their increased expression is not maintained (McMahon *et al*., 1992) and the mesencephalon and rostral rhombencephalon regions (cerebellar anlagen) subsequently fail to develop (McMahon and Bradley, 1990). Thus it appears that the transcription factors En1 and En2 are positional information genes required for the development of the midbrain and cerebellum and that they are initially expressed at the boundary between "head" and "trunk" neuroectoderm and maintained by Wnt1. So what turns on Wnt1 or En1 and En2?

Evidence showing that FGF8 secreted by the isthmus serves this function comes from bead implantation studies in the chick and mutation in zebrafish. Implanting FGF8 soaked beads in more rostral regions of the neuroectoderm induces several genes of the midbrain–rhombomere1 region in adjacent tissue including *Wnt1*, *En2*, and *FGF8*. FGF8 does this by binding to its receptor and initiating a signal transduction pathway that activates Pou2/Oct3/4 transcription factors (Reim and Brand, 2002).

Is FGF8 a morphogen? En2 is expressed in a gradient in the midbrain, an area that forms the optic tectum anterior to the isthmus (at low En2 levels) and the cerebellum posterior to the isthmus (at high En2 levels). This could be due to limited competence of these areas to respond, in which case they are prepatterned, or it could be a graded response to FGF concentration. To test this, the isthmus was grafted to either forebrain or hindbrain regions. When a part of the isthmus itself is grafted to the forebrain, a reversed gradient of En2 is induced nearby, with the higher concentrations near the graft (rostrally) and the lower concentration at a distance (caudally, Fig. 11). In these embryos, an ectopic cerebellar vesicle develops rostral to the ectopic optic tectum, supporting the conclusion that the concentration of the transcription factor En2 is differentially instructive within the development of the midbrain and hindbrain and thus that its inducer, FGF8, can act as a morphogen. However, in the hindbrain location, only cerebellum was induced, indicating that this tissue has received previous patterning information that limits its response to these inductive signals.

Thus the isthmus forms at a boundary between the midbrain (expressing Otx2) and the hindbrain (expressing Gbx2), which for patterning purposes we could say is between the "head" and the "trunk." This interface provides an asymmetrical source of signaling molecules that are involved in AP pattern of the cells both rostral and caudal to it. It is therefore referred to as the isthmic organizer.

## Hindbrain

Just caudal to the isthmus, the neural swelling called the hindbrain or rhombencephalon develops (see Fig. 6). The rostralmost section of this vesicle (r1) expresses En2 in a gradient peaking at the rostral margin (the isthmus) and forms the cerebellum under the influence of FGF8 and Wnt1 (see above). The rhombencephalon is characterized early during development by its subdivision into anatomically identifiable rhombomeres. Rhombomeres 1–7 (r1–r7) form as identifiable bulges in the rhombencephalon proper, and the eighth metameric unit, r8, forms at the caudal end of the visible hindbrain, alongside the first five somites, and is similar in construction to the spinal cord. All eight rhombomeres constitute the rhombencephalon. At their dorsal margin, rhombomeres give rise to neural crest that forms the sensory component of the cranial nerves (along with contribution from ectodermal placodes, see Neural Crest and Placode). Laterally, interneurons form connecting sensory-motor reflex arcs and other inter-CNS connections. Ventrally, they produce motor neurons that contribute to the motor component of the IVth to XIIth cranial nerves. Specific cranial nerves arise from specific rhombomeres (Fig. 12) and cells within the

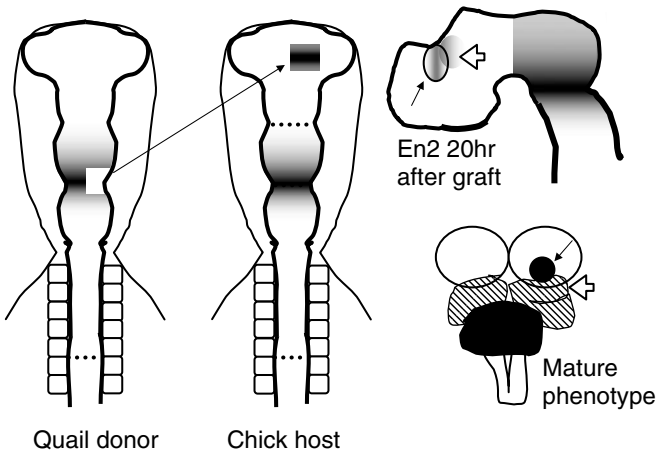


**FIGURE 11.** Gain-of-Function experiment in chick showing the isthmus is sufficient to reestablish the mesencephalon and rostral rhombencephalon when grafted to an ectopic site. Shading indicates the gradient of En2 expression surrounding the isthmus. Neuroepithelium was taken from the isthmus region of a donor quail embryo (empty framed area) and grafted into the prosencephalon (stippled framed area) of a chick host. At 20 hr after grafting, the graft maintained En2 expression (small arrow) and induced En2 expression in the adjacent chick tissue. As with the normal expression, a gradient of En2 expression forms as the distance from the isthmus tissue increases. At later stages, the quail graft contributed directly to an ectopic cerebellum (thin arrow), and chick tissue just caudal to the graft formed an ectopic mesencephalon (open arrow) instead of dorsal thalamus (its normal fate). The ectopic mesencephalon/cerebellum is inverted in the AP plain relative to the host mesencephalon/cerebellum, indicating that their patterning is not influenced by a prepattern within the head neuroectoderm. (Redrawn after Alvarado-Mallart, 1993, Fig. 1.)

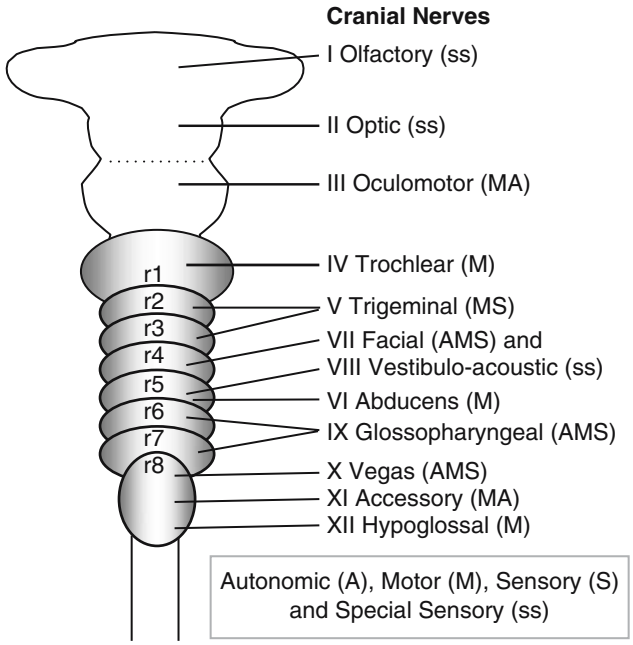


**FIGURE 12.** Cranial nerves: Diagram illustrating the AP origin of each cranial nerve in a d3 avian embryo. Motor and special sensory components come from the neural tube, whereas autonomic and sensory components come from the neural crest and placodes (see also Fig. 17). The motor branch of the trigeminal forms from axons of cell bodies in r2 and r3, and the glossopharyngeal from axons of cell bodies in r6 and r7. Axons contributing to the facial and auditory (vestibulo-acoustic) both exit at the same location in r4 (Lumsden and Krumlauf, 1996).

rhombomeres do not mix between rhombomeres beyond a certain stage. This demonstrates a new feature of patterning not yet addressed here: **segmentation**.

Most of what is known about segmentation and pattern formation was learned from the fruit fly, *Drosophila*. Fruit-fly body segmentation arises by a cascade of gene expression that subdivides a larger field. Large regions are specified by gap genes, and these are further subdivided into two-segment wide regions by the expression of pair-rule genes. Both gap and pair-rule genes are regulated by a morphogen gradient (bicoid) from one end of the embryo. These regions subdivide further under the influence of segment-polarity genes, which establish firm boundaries between the cells of each segment through negative-feedback circuits. As these boundaries are being established, the gap and pair-rule genes turn on specific sets of positional information transcription factors that will determine the later phenotype of each segment. In the fly, many of these positional information genes contain a conserved region called the homeobox. Homeobox-containing genes (*Hom* genes in flies) produce homeodomain proteins that are expressed in overlapping domains and establish positional information based on their rostral boundaries. The order of rostral expression of the *Hom* genes matches their 3′ to 5′ order within the *Hom* gene clusters on the chromosome, a feature called colinearity. *Hom* genes are assisted in their function of generating positional information by two other transcription factors, Extradenticle (Exd) and Homothorax (Hth). Segmentation of the vertebrate hindbrain shares some of these features.

No gap genes have been identified to define primordial subdivisions in the hindbrain as Otx2 and Gbx define the mesencephalic/rhombencephalic boundary and adjacent regions. So in vertebrates this first subdivision of the hindbrain may represent direct responsiveness to combinations of morphogen gradients. This has recently been shown for the normal development of r1, which is patterned by isthmic FGF8 and RA (Irving and Mason, 2000), and for r5 and r6, which depend on a different gradient of RA (Niederreither *et al*., 2000) acting through RAR$\alpha$ or RAR$\gamma$ (Wendling *et al*., 2001). Within the posterior hindbrain many transcription factors are upregulated by the morphogen RA; however, the sources and directions of the RA gradients are a point of contention (Grapin-Botton *et al*., 1998; Begemann and Meyer, 2001).

Although not necessarily involved in a primordial subdivision of the rhombencephalon, some "gaps" or shared qualities are observed between cells in the rostral rhombencephalon and are contrasted with other qualities shared by cells in the caudal rhombencephalon. For example, in humans, the rhombencephalon divides anatomically into metencephalon (which forms the cerebellum and pons and corresponds to the most rostral rhombomeres) and the myelencephalon (which forms the medulla and gives rise to cranial nerves VI–XII). However, this anatomical subdivision is not observed in other model animals. Instead there may be molecular differences between the rostral and caudal rhombencephalon. For example, the cells of r1–r3 differ in their cell division patterns from those in r4–r7/8 (Kulesa and Fraser, 1998) and r1–r4 have a different responsiveness to RA than r5–r8 do (Niederreither *et al*., 2000). Loss of RA signaling results in loss of r5–r8 character and their transformation to r4 identity (Dupe and Lumsden, 2001), whereas increases in RA result in expansion of r4–r8 at the expense of more rostral rhombomeres (e.g., Morriss-Kay *et al*., 1991; Conlon and Rossant, 1992; Niederreither *et al*., 2000). So, although gap genes have not been found in vertebrate hindbrain formation, the concept of larger pattern persists in this region.

In an approximation of the *Drosophila* pair-rule function, the hindbrain is initially subdivided into approximately two-segment units expressing transcription factors later associated with odd-numbered rhombomeres (e.g., Krox20, r3, and r5) and even-numbered rhombomeres (e.g., Hoxa2, r2; Hoxb1, r4; although Kreisler [kr] is expressed in both r5 and r6). At the interfaces between these two-segment regions, asymmetries provide positional information for full segmentation. For example, an analysis of *Krox20* mutant embryos indicates that Krox20 expression between even segments 2/4/6 and odd segments 3/5 is required for appropriate segment formation, cell segregation, and specification of regional identity. (Fig. 13; Voiculescu *et al*., 2001).

The normal formation of boundaries between rhombomeres also depends on the expression of transcription factors Pou2/Oct4 (Burgess *et al*., 2002), and bidirectional signaling mediated by Eph receptors (r3, r5) and their ligands (r2, r4, r6; Klein, 1999). In some ways this is similar to the action of the *Drosophila* segment polarity genes, although the Ephs/ephrins are **realizators** (revealing the cell's fate through their expression) whereas the crucial segment polarity genes are **selectors** (regulating the cell's fate through their expression). In any case, the juxtaposition of these alternating proteins restricts cell mixing *in vitro*, and likely generates the compartment boundaries observed *in vivo* (Lumsden, 1991). Ultimately, each rhombomere is well defined.

As with *Drosophila* segments, each rhombomere also expresses a different set of transcription factors that serve as its positional information (Fig. 14). In vertebrates, as in *Drosophila*, these genes frequently contain a homeobox (*Hox* genes in vertebrates). The order of the rostral boundaries of *Hox* gene expression in the nervous system shows colinearity with their position on the chromosomes. They are regulated by gradients of a morphogen (RA) or morphogens and their function depends on two other transcription factors, Pbx (the homolog of *Drosophila* Exd) and Meis (the homolog of *Drosophila* Hth; Waskiewicz *et al*., 2001). As for being positional identity factors, ectopic expression or repression of these genes causes a shift in rhombomere identity to match the new code.

Thus the segmentation and segment identity cascade first determined in *Drosophila* is mirrored in the vertebrate hindbrain both at the mechanical and molecular level. It is generated through a cascade of signaling within the hindbrain and is autonomous from its surrounding mesoderm. This contrasts with the patterning of the hindbrain neural crest and the spinal cord, which are dependent on signals from the surrounding segmented mesoderm or branchial arches to determine their position.

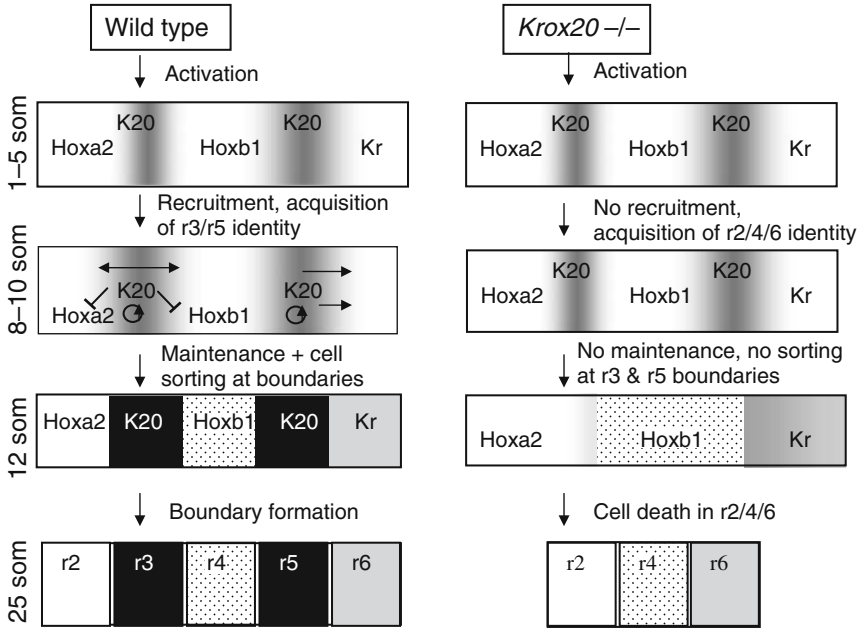


**FIGURE 13.** Model of hindbrain segmentation in mouse using wild-type and *Krox20* mutants. For wild-type embryos, at 1–5 somites, Krox20 is expressed in a few cells at two bands corresponding to prospective r3 and r5. The enhancers for *Hoxa2, -b1*, and *Kreisler (Kr)* are activated. Additional cells are recruited to express Krox20. At the 8–10 somite stage, prospective r3 and r5 express Krox20 homogeneously and recruit cells from adjacent regions (arrows). In addition, Krox20 regulates its own expression (circular arrows) and inhibits the expression of positional information genes from even numbered rhombomeres. By the 12 somite stage, r3 and r5 have acquired their identity. By the 25 somite stage, the rhombomere boundaries are well defined. In Krox20 mutants, the early stages look similar to wild-type embryos. However, the Krox20 regions do not expand or coalesce. Eventually these cells acquire an even numbered rhombomere identity and get incorporated into r2/4/6. By the 25 somite stage, significant cell death has reduced the size of the even-numbered rhombomeres leading to a reduction in the size of the hindbrain. (Adapted from Voiculescu *et al*., 2001, with permission from the Company of Biologists Ltd.)

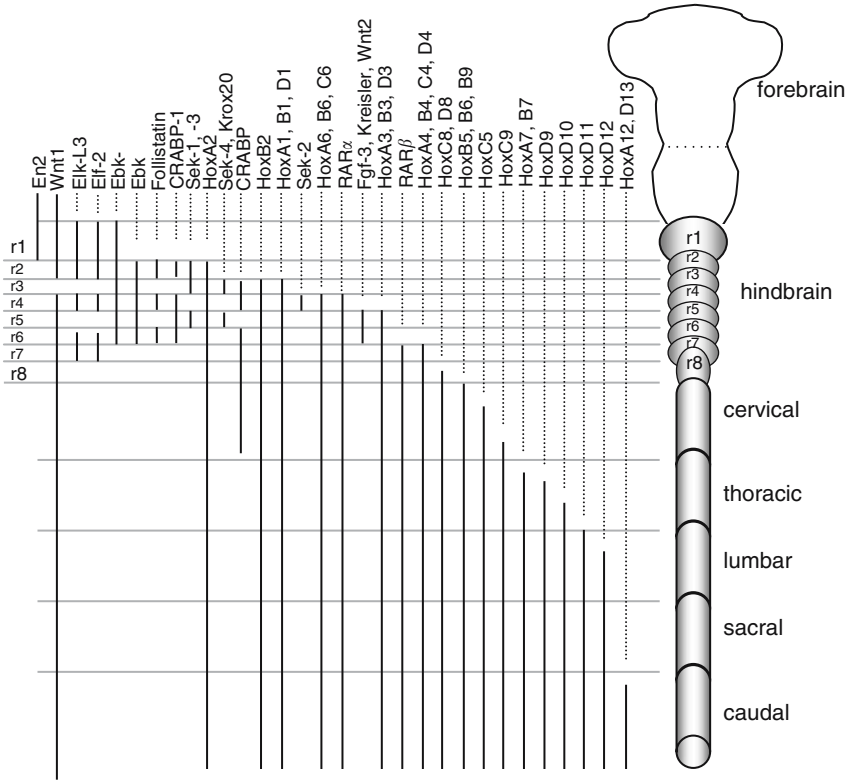


**FIGURE 14.** Diagram of localized gene expression in the developing "trunk." Rhombomere boundaries are specified by specific combinations of transcription factors. In the spinal cord, the rostral limit of *Hox* gene expression delineates positional information.

## Spinal Cord

Colinear *Hox* gene expression is continuous from the hindbrain throughout the spinal cord, with genes located in more 3′ regions of the chromosomes being expressed more rostrally, and those at more 5′ regions in the clusters being expressed more caudally (Fig. 14). These transcription factors provide positional information within the neural tube and adjacent mesodermal somites that controls the development of cervical, thoracic, lumbar, and sacral development in the spine. Evidence in support of this comes from a comparison of the vertebrae of chick and mouse. These two species express similar *Hox* genes in their trunk, and the boundaries of expression of gene pairs match reproducibly with the division between cervical and thoracic (*Hoxc5* and *c6*) and between lumbar and sacral (*Hoxd9* and *d10*) even though these two points occur in different locations in mouse and chick (Fig. 15). In addition, grafting experiments that moved either neural tissue or paraxial mesoderm (somite) to another AP position in the embryo have demonstrated that neural positional information, as measured by AP-level specific motor neuron differentiation, tracks with the level of the adjacent paraxial mesoderm.

At a molecular level, it was anticipated that the mesoderm, which expresses *Hox* positional-information genes and directly underlies the trunk neuroectoderm, would pattern the overlying neuroectoderm directly. Unfortunately, the patterns of expression of the mesoderm and neuroectoderm do not line up. Three mechanisms have been suggested in chick and mouse to account for the observation that positional information genes in the spinal cord do not show the same rostral boundaries in ectoderm and mesoderm. The first possibility is that CNS position is regulated by adjacent paraxial mesoderm to express the same *Hox* genes, followed by differential growth or morphogenesis that would displace the rostral boundaries between these two tissues (e.g., Frohman *et al*., 1990). Alternately, one *Hox* gene in the mesoderm could promote the secretion of signals that would induce another *Hox* gene in the CNS (e.g., Sundin and Eichele, 1992). Finally evidence also exists for the possibility that caudal sources secrete morphogens that form gradients that induce positional genes in the CNS and mesoderm independently, without the requirement for local signaling sources (e.g., Gaunt and Strachan, 1994). Again, it is possible that all of these mechanisms are functioning to regulate different parts of this complex cascade.

The point of establishing a specific *Hox* code within the neural tube is to regulate downstream genes appropriate to particular AP levels of the spinal cord. For example, although generally similar in function, the spinal cord sensory and motor neurons have specific targets depending of their AP level. For example, sensory and motor neurons from the brachial and lumbar regions target the arms and legs, whereas those of the cervical, thoracic, and sacral levels do not. Specific transcription factors, such as the LIM genes in motor neurons are expressed in a distinct pattern within the spinal cord in accordance with their projected targets and due to their *Hox* expression induced by patterning signals from the adjacent mesoderm (Ensini *et al*., 1998).

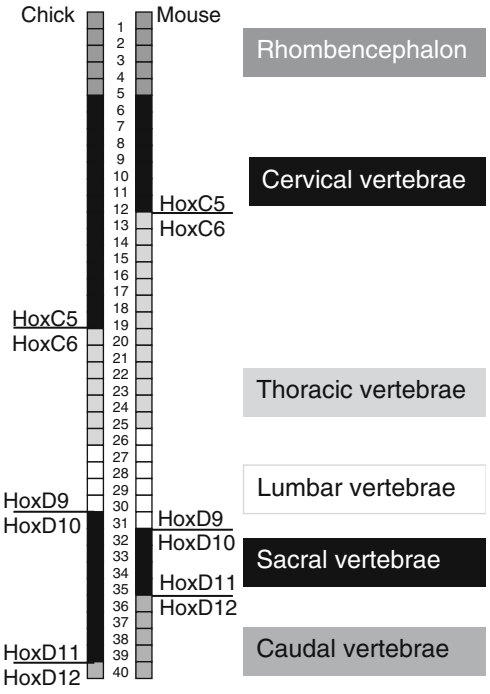


**FIGURE 15.** Specific anatomical boundaries in the mesoderm, for example, between the cervical and thoracic vertebrae, correlate with *Hox* gene expression in the mesoderm. Even though these anatomical transitions do not occur at the same level (somite number). In the chick there are many more cervical vertebrae than in the mouse, but *HoxC6* expression begins in the somite at the level of the first thoracic vertebrae in both species. Numbers down the middle of the figure represent somites.

## Neural Crest

The neural crest cells (see Dorsal Patterning below) are induced at all AP levels of the neural tube except the rostral diencephalon and telencephalon. The regulation of their presence or absence in the AP plane is a function of the same caudalizing and caudal-antagonist signals that promote AP patterning in the CNS. Although no neural crest cells are formed at the boundary between the rostral-most CNS and epidermal ectoderm, treatment of rostral neural ectoderm in *Xenopus* with intermediate levels of BMP and either bFGF, Wnt8, or RA transforms this tissue into neural crest. This transformation can be blocked by expression of dominant negative forms of the appropriate receptor or dominant negative versions of the signal. Similar rostral crest induction can be achieved *in vivo* with the expression of a constitutively active RA receptor (Villanueva *et al*., 2002). These data demonstrate elements of the patterning cascade regulating the no-crest/crest anterior boundary.

Within the crest-forming region, patterning also occurs (Fig. 16). Cells from the anterior crest (of the posterior diencephalon, mesencephalon, and rhombencephalon, down to the level of the fifth somite) form mesectoderm (non-neural cells forming the connective tissues of the cranial muscles and

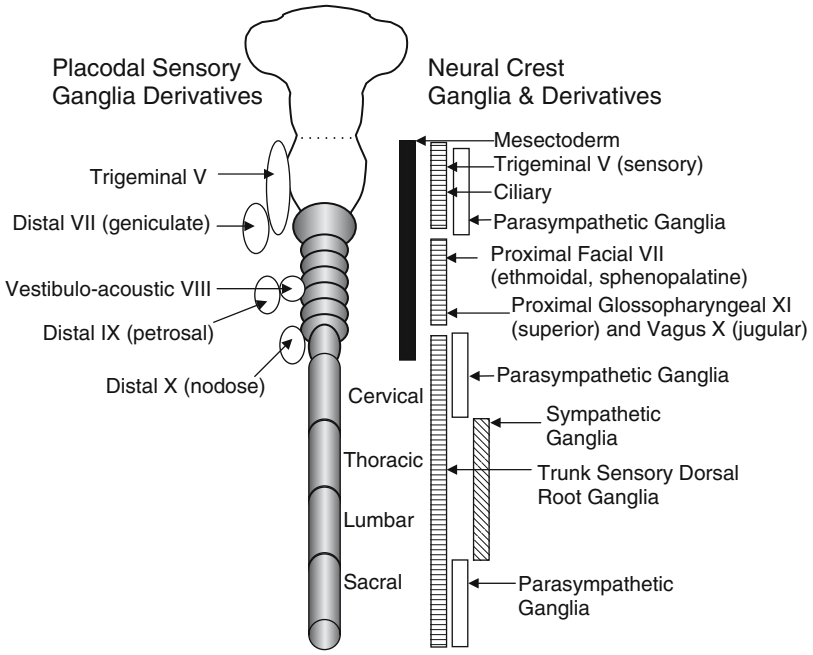


**FIGURE 16.** Placodal and neural crest contributions to the PNS (in part adapted from Le Douarin *et al*., 1993, with permission from Academic Press, Orlando, FL).

the cartilage and membrane bone of the facial skeleton and skull vault), parasympathetic ganglia (cholinergic/Ach-secreting neurons from midbrain and rostral hindbrain levels [r1]), and sensory ganglia (also cholinergic). At spinal cord levels, parasympathetic ganglion cells give way to sympathetic ganglia cells (noradrenergic/noradrenaline-secreting neurons, T1-L2), whereas at the most caudal levels, parasympathetic ganglia reappear (second to fourth sacral segments). Sensory ganglia are formed at nearly all levels of the posterior cranial and spinal neural tube. Grafting studies using chick–quail chimeras, which allow tracking of heterotopically grafted cells to their new fates, demonstrate that all levels of the neural tube have the potential to produce sensory, sympathetic, and parasympathetic neurons from the crest. Therefore, limitations to the pattern must depend on signals independent of CNS patterning.

The understanding of the molecular mechanisms underlying neural crest positional identity is still limited. Many of these mechanisms, such as the involvement of cascades of certain types of transcription factors and lateral inhibition via the Notch-Delta system, have been conserved from our common ancestor with *Drosophila* (Ghysen *et al*., 1993; Jan and Jan, 1993). For neural crest, the extracellular signaling tissues and molecules that control these cascades are still being elucidated. Within the hindbrain region, where crest forms specific cranial nerves associated both with particular rhombomeres and specific branchial arches (and pharyngeal pouches), one can ask if rhombomere positional identity or branchial arch positional identity determines the pattern of these crest cells. Zebrafish mutations that affect the mesendodermal patterning of the branchial arches through which these neural crest cells migrate without affecting the patterning of the rhombomeres indicate that the mesendoderm patterns the crest and not vice versa as had previously been proposed (Piotrowski and Nusslein-Volhard, 2000). In a similar finding based on chick–quail grafting experiments (Couly *et al*., 2002), Hox nonexpressing crest found rostral to the hindbrain were patterned by regional differences in the anterior endoderm (skeletal not neural structures were assessed). Crest from Hox-expressing regions failed to respond to similar signals, again indicating that a prepattern separates cells in the "head" from those in the "trunk."

Emerging evidence indicates that the neural crest choice between sensory and autonomic differentiation hinges on exposure to BMP2 expression in the peripheral tissues, perhaps from the dorsal aorta. *In vitro*, high concentrations of BMP2 initiates expression of the transcription factor MASH1 associated with autonomic differentiation. BMP2 acts instructively rather than selectively. Additional signals from specific AP locations that have not yet been identified could induce the expression of other transcription factors, which act in conjunction with MASH1 to specify the final phenotypes of the different autonomic neuron subtypes (sympathetic, parasympathetic, and enteric). In contrast, in the absence of BMP2, sensory neurons form and express several transcription factors including neurogenin 1 and 2, NeuroD, and NSCL1 and 2 (reviewed by Anderson, 1997).

Although many trunk crest cells are multipotent at the time their migration is initiated and can form either sensory or autonomic (sympathetic) neurons depending on their environment, others may be limited in their potential prior to migration. Trunk neural crest migrating from young neural tubes, which would normally form ventral structures, can differentiate into several cell types including catecholamine-positive (sympathetic) neuroblasts, whereas crest migrating from older neural tubes end up in the dorsal region (presynaptic-sympathetic or sensory

ganglia) and never produce catecholamines. While young and older crest cells can be tricked into migrating to the dorsal or ventral locale that is inappropriate for them, old crest still cannot produce catacholamines (Artinger and Bronner-Fraser, 1992). This demonstrates that some DV pattern is not induced by the migratory environment but involves a cascade that includes changes to the crest that remain in the neuroectoderm layer longer. Perhaps neural crest cell differentiation has a dependence on birth order from a stem cell population as do the cells of the forebrain, where birth order determines the layering of the cerebral cortex.

### Placodes

Placodes are neuroectodermal thickenings that form outside of the boundaries of the CNS and contribute to the paired specialized sense organs (olfactory/nose, optic/lens, otic or auditory/ear, and lateral line system) or to the anterior pituitary gland and cranial sensory ganglia (Fig. 16). Many early marker genes have been identified that are expressed in specific placodes such as *Pax6*, *Otx2*, and *Sox3* in the lens placodes, *Pax6* in the olfactory placodes; *Nkx5.1 Pax8*, and *Pax2* in the otic placodes; *Msx2* and *Dlx3* in the lateral line placodes; *Pax3*, *FREK*, and *neurogenin1* in the trigeminal placodes; and *Pax2* and *neurogenin2* in the epibranchial placodes that form the principal ganglia of the VIIth, IXth, and Xth cranial nerves (see Baker *et al*., 1999 and references therein). However, how these regional specifications are patterned is still a work in progress (reviewed extensively by Baker and Bronner-Fraser, 2001).

In brief, a region of ectoderm competent to form the cranial placodes, the preplacodal domain, forms in the cranial neural plate border region. The expression of several *Pax* (paired-box transcription factor) genes in this ectoderm such that each placodal region expresses a different combination of *Pax* expression (see above). In *Drosophila*, Pax homologs (Ey and Toy) function synergistically with other transcription factors (so) and transcription factor facilitators (eya and dac). Various members of the vertebrate homologs of these transcription regulators, (Six, Eya, and Dach) are expressed with the various *Pax* genes in the placodes, suggesting that a conserved network of genetic regulation may be responsible for establishing specific placodal identity/pattern.

These transcription factors are regulated by signals from various sources. For olfactory placodes the anterior endoderm, prechordal mesoderm, and the anterior neural ridge all have been suggested as sources of inducing signal responsible for activating the appropriate set of transcription factors, although no signal has yet been identified that is either sufficient or necessary for olfactory placode induction. The hypophyseal placode is originally specified by BMP4 from the diencephalon. For lens placode induction, exposure to neural plate and anterior mesendoderm are sufficient, whereas exposure to the optic cup is both necessary and sufficient (via BMP4 and 7). For the trigeminal placodes, an interaction between the neural tube and the surface ectoderm is required to induce the placode but the signal and the method of restricting the placode to a certain location have not been determined. For the lateral line placode, neural plate, axial, and nonaxial mesoderm are each sufficient for induction, and no signaling molecule has been identified. For otic placode formation, evidence points to mesendoderm as the source for an early signal, and to hindbrain as the source for a later signal in a two-step model of early ear patterning. For the epibranchial placodes, pharyngeal pouch endoderm expressing BMP7 is both necessary and sufficient. In summary, placodes are dependent on local environmental signals from various sources to initiate specific sets of highly conserved transcription regulators, which define their fate in the AP plane.

## DV PATTERN

### Ventral Patterning

As mentioned, the formation of the nervous system begins with the induction of a two-dimensional neural plate, which forms in an AP and mediolateral plane across the dorsal surface of the early embryo (Fig. 17). Along its mediolateral axis, polarity is established through asymmetrical signaling from neighboring tissues. At its midline, the neural plate contacts the dorsal mesoderm: the head process and notochord. These tissues formed as ingressed cellular derivatives of the Spemann–Mangold Organizer or node, which is responsible for neural

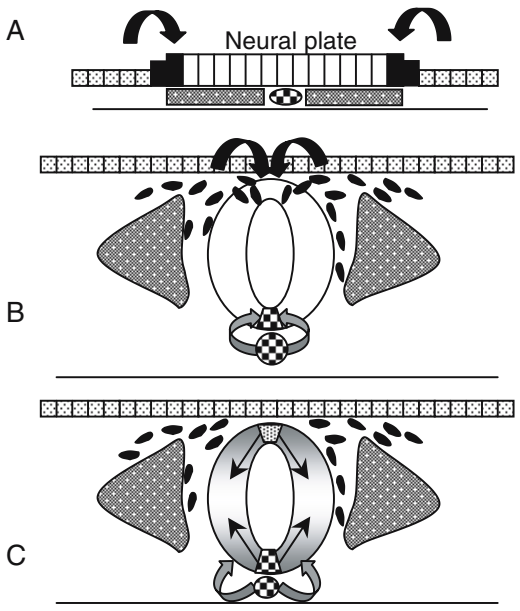


**FIGURE 17.** (A) Neural plate (white) has contact ventrally with the notochord (checkered) and somatic mesoderm (dark stipple), and laterally with the surface ectoderm (stipple). Black arrows indicate the morphogens, BMP-4 and -7 secreted from the surface ectoderm and altering the adjacent cells to form neural crest (black) at intermediate concentration and neuroectoderm (white) at low concentrations. (B) The neural tube is still under the influence of its adjacent tissues. Continued signaling has resulted in the migration of the neural crest (dorsally). Sonic hedgehog signaling (gray arrows) from the notochord induces the formation of a floor plate ventrally (checkered). (C) The induced roof plate (heavy stipple) becomes the new organizing center dorsally, and both the floor plate and notochord continue to secrete the morphogen Shh, which influences ventral patterning.

induction (see Chapter 1). This region becomes the ventral neural tube, but it starts out as the most dorsal (medial), and therefore most neuralized of all the neural ectoderm.

As one of the earliest parts of the neural patterning cascade following neural induction, these medial mesodermal tissues act as an asymmetrical signaling center to pattern the neural plate, and shortly thereafter the neural tube, by secreting a morphogen, Sonic hedgehog (Shh), which was induced in organizer cells and dorsal mesoderm by two transcription factors, goosecoid and HNF-3β (Fig. 18A). Shh sets up the ventral patterning center for the neuroectoderm (Fig. 18B) and orchestrates the specific development of three prospective cell types within the ventral neural tube: the floor plate, the motor neurons, and the ventral interneurons (Fig. 18C). Evidence supporting the cause and effect relationship between notochord, Shh, and ventral cell differentiation within the neural tube comes from several sources. For example, in the normal embryo, immediately adjacent to the floor plate, ventral interneurons (V3) and then motor neurons (MN) develop in response to decreasing levels of Shh signaling, and more lateral still, another type of ventral interneurons (V2) differentiate in response to the lowest levels of Shh (Fig. 18C). In chick, cutting the neural plate to segregate the ventral region from the floor plate or removing the notochord eliminates the formation of MN on the excised side. In addition, loss of the Shh gradient in *Shh*−/−mutant mice results in an expansion of the dorsal phenotypes and a loss of ventral (Fig. 18D). These experiments show notochord and Shh signaling are necessary to induce the ventral pattern of cell phenotypes. In contrast, grafting of an additional notochord at a more dorsal position on the neural tube induces ectopic MN in more dorsal regions (Fig. 18E), showing that notochord is sufficient.

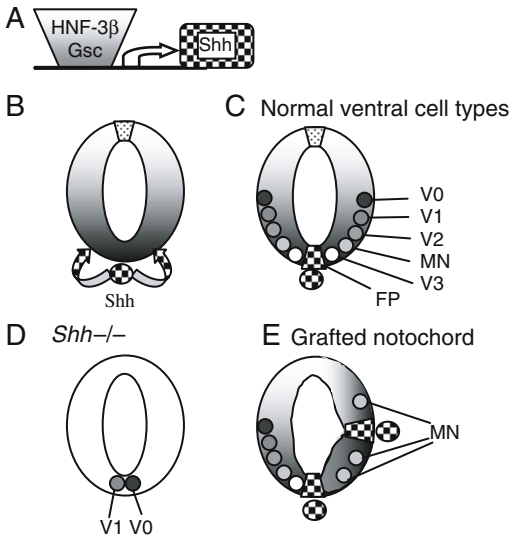


**FIGURE 18.** (A) In the spinal cord, cells of the Spemann–Mangold Organizer and its derivatives express the secreted protein Shh in response to transcription factors HNF-3β and Goosecoid (Gsc). (B) Shh secreted from the notochord and dorsal mesoderm (checkered) establishes a gradient along the ventral to dorsal axis of the neural tube. (C) This signal induces the differential differentiation of MN and four interneuron subtypes (V0–V3) in the ventral neural tube. Genes originally expressed throughout the neural tube, *Pax3* and *Pax7*, are now expressed only in the dorsal region. (D) In mice mutant for *Shh* (−/−), dorsal genes *Pax3* and *Pax7* expand into the ventral region, and ventral cell types are lost, with the exception of two lateral interneuron groups. (E) When a second notochord is grafted lateral to the forming neural tube, an ectopic floor plate and MN are induced nearby. Markers for interneuron were not assessed and the control side is presumed normal with regard to their expression.

A controversy over the induction of floor plate by notochord has been raised (Le Douarin and Halpern, 2000) due to the observation that some notochordless or Shh deficient mutants nonetheless have a floor plate (e.g., see Halpern *et al*., 1993, 1995; Schauerte *et al*., 1998). Studies in zebrafish suggest that the floor plate may be two populations of cells (medial and lateral), the medial being independent of Shh signaling and derived directly from the organizer and lateral being Shh dependent and induced (Odenthal *et al*., 2000). It is unclear if this represents a difference between teleosts and amniotes or is a constant feature of vertebrates. The later is a possibility, since floor plate cells in amniotes can derive directly from the node (Selleck and Stern, 1991; Schoenwolf *et al*., 1992) or be induced in the neuroectoderm by Shh and thus may also represent two populations. Regardless, the floor plate cells express Shh, either through induction or as a direct derivative of the organizer.

The expression of Shh by the floor plate contributes to the morphogen gradient of Shh in the ventral neural tube and maintains it once the notochord has moved away from the ventral neural tube. But what is the molecular mechanism for ventral neural patterning? Studies of the hedgehog signal transduction pathway in *Drosophila* indicate hedgehog ligands work through a twelve-pass transmembrane receptor called Patched (Ptc) when it is bound to Smoothened (Smo), a G-protein-coupled transmembrane protein. Ptc constitutively inhibits signal transduction by Smo, and hedgehog binding lifts that inhibition. Smo uses a signal transduction pathway involving Protein Kinase A (PKA) and activates a Gli-family transcription factor (reviewed in Litingtung and Chiang, 2000). In vertebrates, two Ptc and three Gli homologs have been identified with appropriate expression localization. The Ptc homologs have high Shh binding affinity and the ability to form a complex with vertebrate Smo. Constituitively active vertebrate Smo mimics high Shh activity in the neural tube, and vertebrate Glis can be responsive to PKA. When activated, Gli proteins bind to Shh responsive promoters linked to a reporter gene and they ectopically induce ventral cell fates (reviewed in Litingtung and Chiang, 2000). This and other evidence indicates that this model pathway (Fig. 19) is probably conserved between flies and vertebrates.

What are the results of this signaling pathway to the patterning of the ventral neural tube? Shh signaling initially upregulates Pax6 and downregulates Pax3 and 7 in the ventral neural tube. Within this ventral Pax6 territory, Shh patterns the neural tube in two steps: first by inhibiting the transcription of certain transcription factors (Dbx1, Dbx2, Irx3, and Pax6; known as Class I transcription factors) in the ventral neuroectoderm in a concentration-dependent fashion, and second by inducing appropriate ventral transcription factors (Nks2.2 and Nkx6.1; known as Class II transcription factors) in these cells (Briscoe *et al*.,

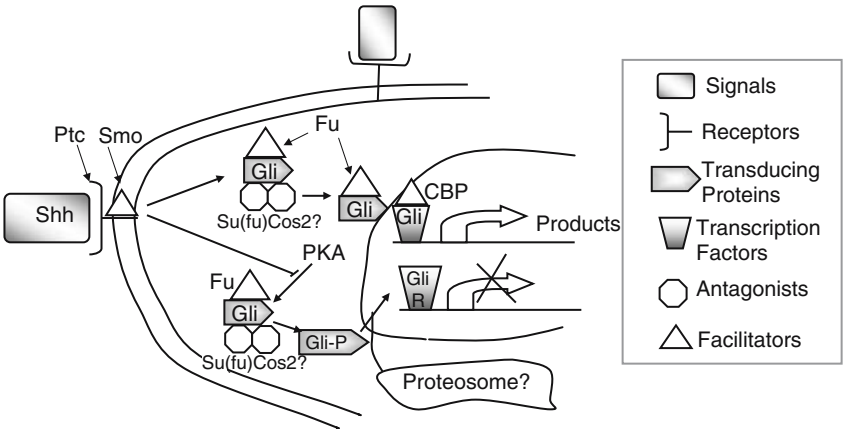


**FIGURE 19.** Signal transduction by Shh. Shh binds to receptor Patched (Ptc), which is associated with the membrane bound signal transduction facilitator Smo. Smo activates cytoplasmic Gli, which in conjunction with the cytoplasmic facilitator fu (if not antagonized by Su(fu)) can relocate to the nucleus. There Gli can cooperate with the nuclear facilitator CBP to activate ventral target genes including *Ptc*, *Gli* and *Shh*. A different pathway involving PKA allows Gli to act as a repressor (GliR) on dorsal genes such as *Pax3* and *Pax7*. Smo and Ptc activation by Shh can also block Gli repressor (GliR) formation, possibly by inhibiting the formation of phosphorylated forms of Gli (Gli-P). The efficient processing of Gli may require phosphoryletion and be proteosome dependent. The vertebrate homolog of Cos2 has not been identified; however, in *Drosophila*, Cos2 binds to antagonizes Shh signaling. (Adapted from Litingtung and Chiang, 2000, *Dev. Dynam.* Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

2000; Fig. 20). Class I and II transcription factors negatively regulate one another's gene expression to create clear boundaries between the progenitor domains. The specific combination of transcription factors in a given region then provides the DV positional information required to differentiate as the appropriate cell type for that region. For example, prospective MN neurons express Nkx6.1, while prospective V2 neurons express both Nkx6.1 and Irx3. Ectopic expression of Irx3 in prospective MN neurons changes their differentiation to V2 fate (Briscoe *et al*., 2000). Similar gain and loss of function experiments indicate that the other cell types are also regulated by the specific combinatorial expression of these genes.

However, the Shh-neural story is more complicated than this. For example, through a pathway independent of the Ptc–Smo–Gli pathway, Shh may mediate adhesion of the neural tube and allow migration of neural crest from the dorsal neural tube where Shh concentration is minimal (Testaz *et al*., 2001). Second, Shh has also been implicated as a mitogen in the neural tube (e.g., see Britto *et al*., 2000), and differential growth is another aspect of pattern formation not considered here. Third, other intracellular and extracellular factors are known to facilitate or limit Shh activity or diffusion (reviewed by Capdevila and Belmonte, 1999; Robertson *et al*., 2001), further regulating the activity of this morphogen. For example, Ptc the Shh receptor, in the absence of Smo acts as a Shh sink and limits its diffusion. Fourth, some ventral phenotypes do develop in the absence of Shh (V0, V1), and these can be induced in neural explant culture by RA; (Pierani *et al*., 1999), a morphogen secreted by the paraxial mesoderm. Thus, other morphogens and signaling sources may also participate in the patterning of the ventral neural tube. Finally, the double *Shh*:*Gli3* mutant mouse has MN; thus there has to be some other induction path for MN that is normally inhibited by Gli3 in the absence of Shh (Litingtung and Chiang, 2000).

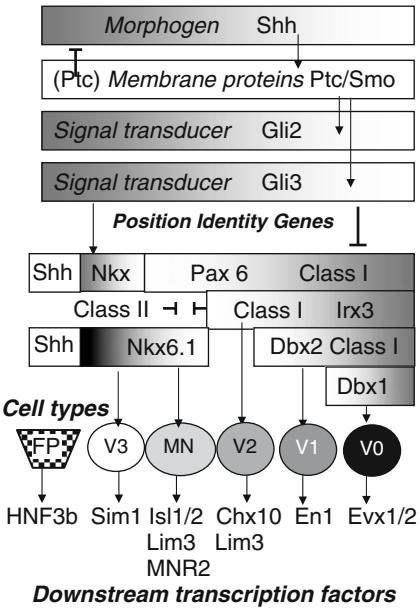


**FIGURE 20.** Shh induction of specific ventral cell fates. Shh, a morphogen, acts through receptor Ptc and its binding partner Smo to activate signal transducers Gli2 and Gli3. Gli activity gradients may result from differential transport of this protein into the nucleus. Gli may regulate both Class I (*Pax6*, *Irx3*, *Dbx2*, *Dbx1*) and Class II (*Nkx2.2*, *Nkx6*) genes. Class I genes are position identity genes expressed in a gradient with their highest level dorsally, whereas Class II gene gradients have their highest concentration ventrally. Thus Gli2 and 3, with their ventral gradient, could inhibit Class I genes and activate Class II genes. By combinatorial effect, the expression of these transcription factors establishes progenitor domains and results in the expression of specific downstream marker genes. In this case, these are also transcription factors that help determine the fate of these cells. (Adapted from Litingtung and Chiang, 2000, *Dev. Dynam.*, 2000. Reprinted by permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc.)

## Dorsal Patterning

Whereas Shh is a positive morphogen for the medial neural plate and ventral neural tube, several members of the TGF$\beta$ family of growth factors (including BMPs and GDFs) are positive morphogens for the lateral, and later dorsal, neural ectoderm, which includes neural crest, roof plate glia, and three dorsal interneuron types (D1A, D1B, and D2; Fig. 21; Lee *et al*., 1998). BMPs (4 and 7) are expressed in the epidermal layer and initially act as morphogens such that the highest levels induce epidermal ectoderm, intermediate levels induce neural crest, and lower levels induce neural plate. BMPs are sufficient to upregulate the same genes (*Pax3, Pax7*) in the lateral neural plate and dorsal neural tube that Shh represses in the medial/ventral region. Thus it appears that these two morphogens are working as opposing gradients to pattern in this plane (like the Saxen–Toivonen model in AP pattern, see Fig. 7C). As these dorsalized *Pax* genes originally were expressed throughout the neural plate, their expression is not sufficient to initiate the cascade that will result in the patterning of the dorsal neuroectoderm. However, their expression is required for appropriate differentiation of the most dorsal cells, the neural crest.

Neural crest cells are the migratory founding cells of much of the PNS (see Fig. 5). They form at the margin of the epidermal ectoderm and neural plate (the neural fold) in response to required signals from both these tissues. However, they are not committed at the neural plate stage, as individual cells can contribute to crest, epidermis, or dorsal CNS. Induction of neural crest appears to be a multistep venture involving early Wnt signaling and later BMP signaling (Bronner-Fraser, 2002). Other positive factors involved include FGF (Lee and Jessell, 1999); Zic2, a zinc-finger transcription factor that promotes crest and inhibits neural differentiation (Brewster *et al*., 1998); FoxD3, a winged-helix transcription factor that works with Zic2 in determining crest (Sasai *et al*., 2001); and Noelin-1, a secreted factor that is induced in a gradient in the dorsal neural tube and provides a competence factor to the dorsal cells, allowing them to differentiate as crest (Bronner-Fraser, 2002).

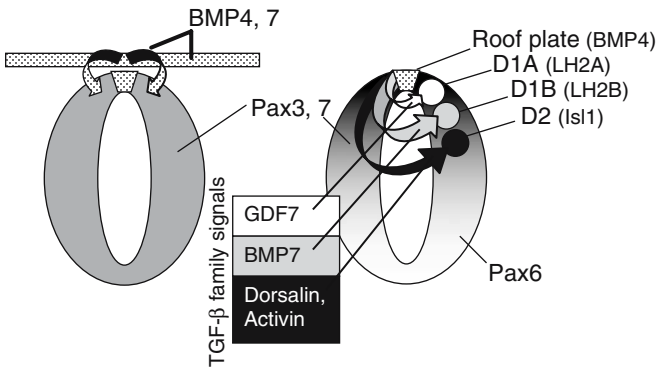


**FIGURE 21.** Dorsal neural patterning. Signals from the overlying surface ectoderm (BMPs) induce the roof plate (stipple), which also expresses BMPs. *Pax3* and *-7* shift from a pattern of expression throughout the neural tube to a concentration in the dorsal neural tube, whereas *Pax6* gets upregulated in the ventral neural tube. Several signaling molecules are secreted from the roof plate, and these induce the fates of several dorsal interneurons (D1A, D1B, and D2), which subsequently upregulate the expression of cell-type specific genes (*LH2A*, *LH2B*, and *Isl1*).

Just as Shh induces Shh secretion from the floor plate to assist in ventral patterning, exposure to secreted BMPs from the surface ectoderm also induces the expression of BMPs and other secreted factors in the adjacent ectoderm. This homeogenetic induction maybe necessary to maintain an accurate gradient within the neural tube as it grows and becomes separated from its neighboring tissues. The difference between the ventral and dorsal patterning is, rather than acting as morphogens, many of the dorsal signaling molecules (Noelin-1, several Wnts, Dsl1, BMP4, and BMP7) seem to regulate the differentiation of specific targets, and thus are secondarily involved in dorsal patterning (Fig. 21). For example, Noelin-1 is specifically involved in neural crest formation, whereas Wnt-1 and Wnt3a (Muroyama *et al*., 2002) or TGF-family (BMP4, 5, 7, GDF6/7, and DSL1; Liem *et al*., 1997; Lee *et al*., 1998) expression in the roof plate provides the signal to induce the dorsal-most interneurons, D1A, and D1B. The more ventral D2 is induced by activin (Liem *et al*., 1997).

### Signal Transduction of BMPs in Dorsal Patterning

BMPs are the initial signaling molecules of dorsalization, and they require receptors and signal transduction pathways to initiate the expression of the other signaling molecules they induce. BMP 4 and 7 act as dimers and bind to serine/threonine kinase receptors (BMPRI, BMPRII, Alk8, tolloid, BMP2b/swirl, snailhouse, somitabun). These activate SMAD proteins intracellularly, which migrate to the nucleus and act as transcription factors for BMP-activated genes (Fig. 22). These are commonly used signaling pathways and a large number of signal transduction modifiers have been identified including other Smads (6 and 7), transcriptional activators (p300), and transcriptional repressors (Ski, Tob). These apparently allow this signal transduction pathway to regulate specific sets of target genes in a given tissue. In the zebrafish model system, reduction in BMP function through mutation of SMAD 5 (somitabun) causes an expansion of dorsal neural phenotypes into the epidermal ectoderm, while loss of one BMP directly from mutation results in loss of both epidermal ectoderm and dorsal neural phenotypes (Fig. 23), showing this pathway is both necessary and sufficient for dorsalization.

## DV Pattern at Other AP Levels

Obviously, not all levels of the neural tube form the same types of neurons as the spinal cord, thus one must consider what mechanisms account for these differences in DV pattern at other AP levels. One could imagine that other responses in the DV plane might stem from intrinsic differences in the AP character of the neural plate. Alternately, one might imagine the differences stemming from differences in the localization of tissues that provide the morphogens and thus from morphogen availability. As usual, evidence exists for both.

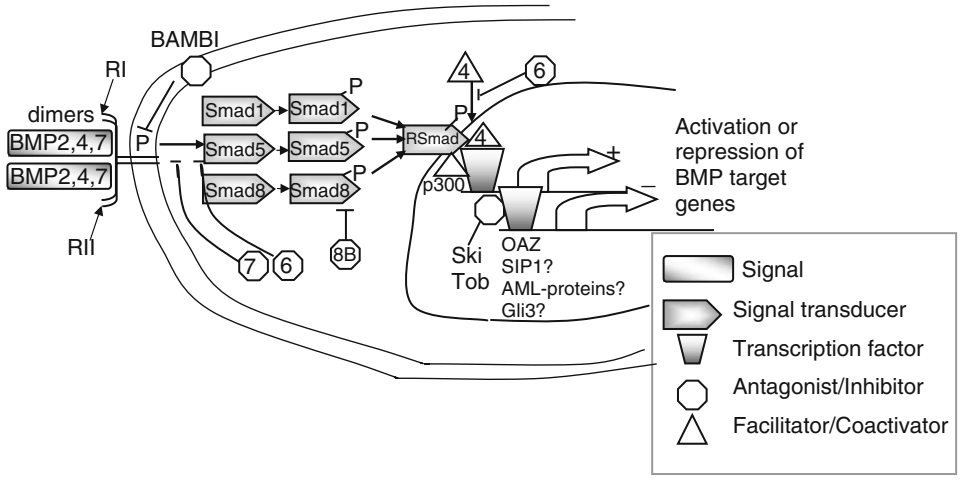


**FIGURE 22.** The main BMP–Smad signal transduction pathway. BMP2, 4, or 7 dimers bind to a receptor complex of type I and type II receptors (RI and RII). This leads RI to phosphorylate RII, which then phosphorylates Smad1, -5, or -8, depending on the cell. This allows this Smad to form a complex with the facilitator Smad4 and this complex enters the nucleus to bind to the MH1 domain and activate or repress target genes depending on which other facilitators or antagonist factors are present in the nucleus. Several nuclear factors also regulate this pathway by acting as transcription facilitators or antagonists. General facilitator p300 can bind to the MH2 domain of Smad1 and -4 and activate transcription through its histone acetylase activity. Ski and Tob act as antagonists to this pathway by binding to various Smads. Smads activate transcription by binding to other transcription factors. Transcription factors that can bind to Smads include OAZ, SIP1, AML, and Gli (C-terminally truncated). Although they are shown as heterodimers, the stoichiometry between Smad1, -5, -8, and Smad 4 is unknown. Smad6, -7, and -8B inhibit this transduction pathway cytoplasmically or in the nucleus, whereas BAMBI is a membrane bound antagonist. Smad6, -7, BAMBI, and Tob are all products of this pathway, leading to negative feedback loops. (Redrawn and adapted from von Bubnoff and Cho, 2001, with permission from Academic Press, Orlando Florida.)

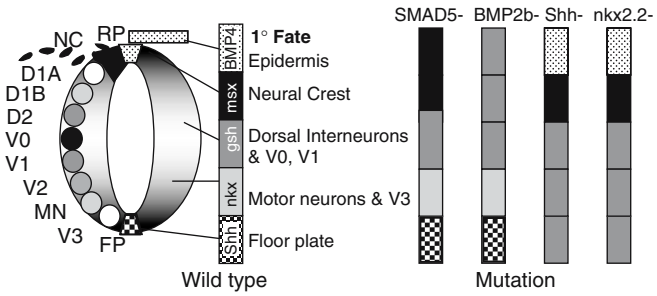


**FIGURE 23.** A summary of DV patterning in the vertebrate neural tube showing a schematic of the neural tube and the specific cell types that differentiate at various DV levels to the left, and the primary fate of various DV levels to the right. To the far right, the results of various mutations are shown. The *SMAD5*-mutation results in low BMP signaling, and the epidermis, which requires high BMP signaling, is replaced with neural crest cells. *BMP2b*-mutants (very low BMP signaling) replace both dorsal tissues with a dorsal interneuron phenotype. *Sonic hedgehog* mutations, in contrast, cause a shift in ventral cell types similar to the loss of *nks2.2*, which is required for MN-differentiation and some ventral interneurons. (Adapted from Cornell and Von Ohlen, 2000, with permission from Elsevier Science.)

Around the isthmus, Engrailed-2 (En2), a transcription factor required for normal cerebellar development, is expressed in the neural tube at all DV levels except the floor plate (Fig. 24A). When the notochord, which expresses Shh, is surgically removed, En2 expression expands into the ventral midline (Fig. 24B). When an ectopic Shh secreting notochord or floor plate is grafted adjacent to the neural tube at more dorsal levels, En2 expression is suppressed (Figs 24C, D). Also at the midbrain level and immediately adjacent to the floor plate, dopaminergic rather than MN form. Dopaminergic neurons are not found at hindbrain or spinal cord levels. Why? Transplantation studies indicate that AP pattern limits the differential competence of ventral neuroepithelial precursors. The same is true for ventral genes in the forebrain. Shh upregulates *Nkx2.1*, which is needed in the diencephalon to form the hypothalamus, but only in the regions where AP patterning gene *Six3* is expressed (Kobayashi *et al*., 2002). Shh also regulates *Pax* genes in the ventral brain, although their expression patterns differ somewhat from those in the spinal cord. In the head Pax3 and Pax7 are pushed far dorsally and are segregated from Pax6, which is also excluded from the most ventral region of the tube, restricting it to the area between the sulcus diencephalicus medius and ventralis. These examples all support the idea that Shh is a common ventral morphogen, but the response depends on previous changes wrought by the patterning cascade at each AP level of the neural tube.

The model in which the DV morphogens differ at various AP levels is also supported in some cases. For example, within the head a new dorsal signaling factor has recently been identified (in *Xenopus*), Tiarin, which dorsalizes the anterior neural plate, causing expansion of dorsal and suppression of ventral neural markers (Tsuda *et al*., 2002). In addition, at the level of the rostral diencephalon an old morphogen, BMP7, is expressed in a new location: the *ventral* midline mesendoderm. At this AP level no floor plate forms and a different set of ventral marker genes including *Nkx2.1* is upregulated due to the inductive signals of both Shh and BMP7. Likewise, whereas an intermediate concentration of BMP induces neural crest as far rostrally as the mid

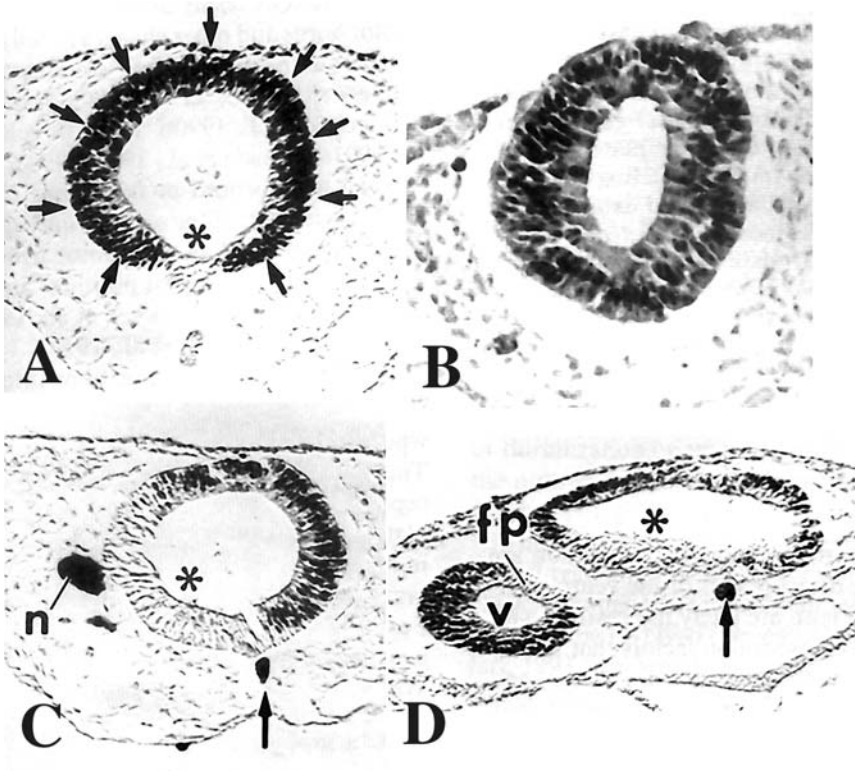


**FIGURE 24.** Engrailed-2 expression in the chick (stage 12). (A) Expression (arrows) in the normal embryo is high at all DV levels of the mesencephalon and rostral metencephalon, with the exception of the floor plate (asterisk). (B) In embryos in which the precursors of the notochord have been removed, no notochord and subsequently no floor plate form, and En2 is expressed uniformly at all DV levels of the neural tube. (C) Grafting of a notochord (n) to a position lateral to the neural tube results in a loss of En2 expression adjacent to the graft (asterisk), showing that notochord is sufficient to inhibit En2 expression. (D) Similar grafting of a fragment of the neural plate containing a floor plate (fp) results in similar suppression (asterisk). (Darnell and Schoenwolf, 1995; Darnell *et al*., 1992) (Developmental Dynamics, and Journal of Neurobiology. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

diencephalon, rostral to this, no neural crest is formed even though BMP is present in the epidermal ectoderm. A number of experiments have implicated caudalizing FGFs and Wnts as the requisite additional morphogens required to induce neural crest at appropriate levels, and a recent study has supported these and added RA to the list (Villanueva *et al*., 2002). Thus, both AP differences in signal availability and AP differences in responding cell competence can shape the intersection between AP and DV pattern.

## LEFT–RIGHT ASYMMETRY

Although significant recent progress has been made on the signaling cascade that confers left–right asymmetry on the early embryo, controlling heart looping and gut rotation (reviewed in Mercola and Levin, 2001), no connections have been made with the left–right asymmetries of the adult brain. These asymmetries do not correlate with known left–right (LR) patterning in the early embryo. For example, people with situs inversus, a condition in which the body LR axes are reversed such that their hearts are angled toward the right and their livers are on the left, still process language on the left side of their brains, as do 95% of people with normal LR patterning. Studies of mirror-image identical twins (and conjoined twins) have lead to speculation that the mechanism for LR patterning in the head is separate from the patterning of the trunk just as many other aspect of head and trunk patterning are independent.

## CONCLUSIONS

The predominant method of achieving a patterned vertebrate nervous system involves responses to signaling from an asymmetrical source. This instigates the activation of new transcription factors in the responding tissue, which leads to a cascade of cellular changes that generate additional asymmetry and cell differentiation. Several of the signaling sources have been identified, including the neural organizer/node, the anterior neural ridge, the isthmus, and the caudal mesoderm along the AP axis, and the notochord and epidermal ectoderm in the DV axis. Signals induce changes in target cells depending on concentration, and antagonists or distance protect other cells from responding, generating diverse cell types. These general concepts and in many cases the specific genetic networks for patterning have been conserved for hundreds of millions of years.

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