



Patterning the Vertebrate Neuraxis

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Neuraxial patterning is a continuous process that extends over a protracted period of development. During gastrulation a crude anteroposterior pattern, detectable by molecular markers, is conferred on the neuroectoderm by signals from the endomesoderm that are largely inseparable from those of neural induction itself. This coarse-grained pattern is subsequently reinforced and refined by diverse, locally acting mechanisms. Segmentation and long-range signaling from organizing centers are prominent among the emerging principles governing regional pattern.

The central nervous system (CNS) arises from the neural plate, a cytologically homogeneous sheet of epithelial cells that forms the dorsal surface of the gastrula-stage embryo. The peripheral nervous system arises from ectodermal placodes and neural crest cells that form at the lateral fringes of the plate. The neural plate subsequently rolls up on its anteroposterior (AP) axis to form a tube, the expanded anterior end of which then partitions into a series of vesicles representing the anlagen of fore-, mid-, and hindbrain. Posteriorly, the long, uniformly narrow tube forms the spinal cord. These early morphological features of the neuraxis, accompanied by position-specific expression of developmental control genes, dictate the overall plan of the CNS and predict its regional specializations. Within each region, a large diversity of neuronal cell types is then generated, each with distinct identities in terms of morphology, axonal trajectory, synaptic specificities, neurotransmitters, and so on. The intricate spatial order of differentiated neurons, essential to the subsequent formation of functional circuits, is crucially dependent on correct regional specification.

Signals from adjacent tissues are involved at all stages of neuraxial patterning. Neural-inducing factors and modifiers produced during gastrulation by the (endo)mesoderm establish an initial crude AP pattern in the overlying neural plate. Although the precise nature of this early patterning information remains unclear, the inductive signals that confer forebrain identity appear to differ qualitatively from those that operate more posteriorly. The coarse-grained pattern that emerges at the end of gastrulation

is progressively refined, resulting in a precise regional variation in cell identity.

Patterning of cell types appears to be organized on a Cartesian grid of positional information, the coordinates of which correspond with the AP and dorsoventral (DV) axes of the neural tube: analyses of cell fate after experimental rotation of the neural plate (1, 2) have indicated that regional fate is determined along the AP axis before and independently of fate restriction on the DV axis. We will confine our discussion to the assignment of AP regional identity, as patterning on the DV axis has been comprehensively reviewed elsewhere (3, 4). We will focus on two well-studied regions, the hindbrain and the midbrain, to illustrate distinct but not mutually exclusive modes of local patterning: segmentation plays a prominent role in the hindbrain, whereas a discrete signaling region at the isthmus sets up the AP polarity of the midbrain. The forebrain has been less intensively studied, but interesting parallels with hindbrain development are considered.

Early Role of the Mesoderm in Regionalization

Investigation of the earliest developmental events has focused on the amphibian embryo, in which it was first seen that neural fate is imparted to competent ectoderm by signals emanating from the dorsal blastopore lip. Spemann noted that the early dorsal lip, grafted heterotypically, could induce an entire neuraxis but the later dorsal lip could induce only posterior CNS (5). Although the details of how the dorsal lip (Spemann's organizer) confers polarity on the neuraxis are still unknown, emphasis has been placed on the posterior-to-anterior (P-to-A) progression of mesoderm involution during gastrulation and a two-step, activation-transformation action of its signals on the dorsal ectoderm. Activating signals from early-involuting mesoderm are

thought to induce a default state of anterior neural differentiation, which is then modified to a more posterior character by transforming signals from later-involuting mesoderm (6). The level of transforming signal impinging on any one level of the neuraxis might confer its local identity (Fig. 1). Inducing signals could pass to the ectoderm either vertically, from underlying cells, or tangentially, from organizer cells still in the plane of the ectoderm (7, 8).

Whereas the molecular identity of activating signals remains uncertain, three secreted proteins expressed by the organizer—follistatin, noggin, and chordin—are capable of inducing the expression of anterior neural plate markers in naïve ectodermal cells (9). These candidate activators share no obvious structural features, but each can bind directly to and antagonize the actions of members of the BMP family of signaling molecules (10, 11). Induction of anterior neural plate may thus involve inhibition of the neural inhibitors BMP2 and BMP4, which are present in the presumptive neurectoderm.

Candidate transforming signals include basic fibroblast growth factor (bFGF) (12) and retinoic acid (RA) (13), both of which can posteriorize anterior neural tissue but have little neuralizing capacity on their own. However, signaling through the bFGF receptor is not necessary for this process; when blocked in transgenic *Xenopus* embryos by the expression of a truncated, dominant-negative form of FGFR1, the posterior CNS forms normally (14). Strongly indicating a posteriorizing role for RA, however, the expression of a constitutively active retinoid receptor results in a posteriorized axis, whereas dominant-negative retinoid receptor expression results in an anteriorized axis (15). The posteriorizing role of RA will be considered again in the context of local patterning in the hindbrain.

In addition to signals that may influence pattern along the entire neuraxis, there is also evidence for head-specific induction pathways. The *Lim-1* and *Otx-2* homeobox genes are expressed in the organizer region (Hensen's node of amniote embryos), and their inactivation in mice deletes all head structures, including prosencephalon, mesencephalon, and anterior hindbrain, whereas the posterior hindbrain and spinal cord are unaltered (16–18). The requirement for these genes appears to distinguish early organizer functions from later ones. Both genes are later expressed in the prechordal plate (16, 19), emphasizing the importance of this structure in endowing the overlying forebrain with unique characteristics. A recently identified secreted protein, cerberus, has potent forebrain-inducing activity (20).

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Cerberus is abundantly expressed in the deep-layer cells of the organizer that constitute the leading edge of the gastrulating endomesoderm. Both the maintained expression and inducing activity of cerberus would seem to depend on coexpression of other organizer factors. It seems likely that *in vivo* forebrain-inducing activity lies in the prechordal plate and the endomesoderm immediately anterior to it, where expression of cerberus and chordin overlap (20). The different inductive activities of early and later dorsal lips, first recognized by Spemann, are now being dissected at molecular and genetic levels; it appears that the acquisition of coarse-grained pattern along the neuraxis is controlled by mechanisms that differ between the anterior-most (prechordal) and the more posterior (epichordal) regions of the neuraxis.

Hindbrain—a Segmented Region of the Neuraxis

Pronounced axial variation involving a comparatively small repertoire of cell types makes the hindbrain an attractive and accessible system for the study of local CNS pattern. Furthermore, early development of the hindbrain is characterized by metamerism, suggesting the early allocation of defined sets of precursor cells and the existence of precise boundaries to both cellular assemblies and realms of gene action. In the chick embryo, the segmented pattern of the hindbrain emerges upon neural tube closure as a series of bulges—rhombomeres—and is virtually complete at the onset of neurogenesis. Segmentation of the vertebrate hindbrain bears a superficial resemblance to segmentation of the *Drosophila* embryo: rhombomeres form by internal subdivision rather than by budding from a growth zone, and

they have a pair-wise organization (21).

Neuronal pattern. Two patterns of metamerized cellular organization can be distinguished in the hindbrain, one involving neurons of the reticular formation and the other involving motor neurons (Fig. 2). Eight identified types of reticular neuron are repeated through sequential rhombomeres such that each contains a more or less complete set (22). Motor neurons also develop in each rhombomere, but have rhombomere-specific identities (23, 24). The segmental disposition of branchiomotor nuclei in the early hindbrain has a close anatomical (23) and functional (25) correspondence with target structures associated with the segmented series of branchial arches that lie beneath it. Later in development, the segmental origins of these cells become obscured as certain reticular cells become more numerous in particular rhombomeres (26) and the motor nuclei condense and migrate to new positions. Fate-mapping studies have also revealed metamerized origins for the adult sensory nuclei (27). In the hindbrain, segmentation is involved in specifying the pattern of developing structures, but not in deploying them in the adult.

Compartment-like properties of rhombomeres. Developmental compartments provide a way of allocating blocks of cells with distinct properties (28). The containment of polyclonal assemblages of neuroepithelial cells within rhombomeres has been shown by lineage-tracing studies in chick (29). Compartmental restriction of cell mingling persists while the epithelium is predominantly germinative (30); later, young neurons may escape the restriction once they have acquired their ultimate positional specification. Rhombomeric domains of the germinative (ventricular) zone

remain lineage-restricted up to late stages, when neurogenesis is nearing completion (31).

Rhombomeres partition from one another according to an adhesion differential that displays a two-segment repeat (Fig. 3) (32). Consistent with an expected tendency of neighboring cell groups to separate, enlarged intercellular space is the earliest specialization of rhombomere boundaries (33). Complementing this adhesive differential is an alternating periodicity to the expression domains of the Eph-like receptor tyrosine kinases and their ligands (Fig. 4) (34–36). Perturbing Rtk-1 (Sek-1) function in zebrafish and *Xenopus* embryos by expression of a dominant-negative form of the mouse Sek-1 receptor results in failure to establish sharp inter-rhombomere boundaries (37). These ligand-receptor partners may thus mediate repulsive interactions that serve to

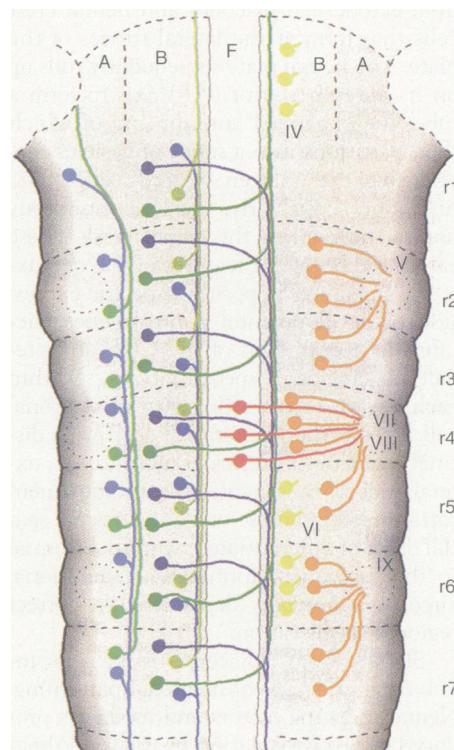


Fig. 2. Patterns of cell organization in the 3-day embryonic chick hindbrain. Superimposed on the rhombomere pattern (r_1 to r_7) are the reticular neurons (left side) and the motor neurons (right side). Reticular neurons are classified (and colored blue or green) according to axonal trajectory. Motor neurons (in the right side basal plate, B) are classified as somatomotor (yellow), innervating extrinsic eye muscles (IV, troclear; VI, abducens); branchiomotor (orange), innervating branchial muscles in the first arch (V, trigeminal), second arch (VII, facial), and third arch (IX, glossopharyngeal); and vestibuloacoustic efferents (red), which share the VII^{th} nerve exit point (dotted circle) with the facial motor neurons in the alar plate (A). F, floor plate.

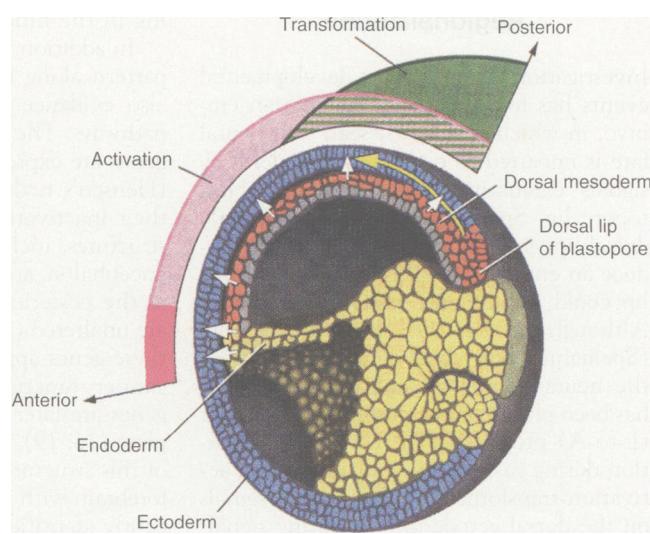


Fig. 1. Acquisition of AP pattern during neural induction in *Xenopus*. In the late gastrula, shown in hemisection, involuted cells have reached the anterior pole of the presumptive CNS. Radial signals (white arrows) from the leading-edge endoderm (yellow) and the mesoderm (orange) induce neural fate in the overlying ectoderm (blue). Forebrain (dark pink) is induced by leading-edge endoderm and mesoderm. More posterior levels of the ectoderm are activated (light pink) and transformed by a graded posteriorizing activity (green). The yellow arrow shows the route of planar signals.



sharpen rhombomere borders. They may also provide a potential mechanism whereby cells in adjacent rhombomeres interact with each other to establish additional cell states at the inter-rhombomere boundaries (21, 33, 38–41). Finally, the inter-rhombomere boundaries become colonized by axons, perhaps on account of both the local expression of growth-promoting molecules (23) and the availability of extracellular space (33).

Hox genes encode positional value along the AP axis. Prime candidates for conferring rhombomere identity are the clustered homeobox-containing genes of the Hox family (42), homologs of the HOM-C genes that encode parasegment identity in *Drosophila*. Expression of genes at the 3' ends of the Hox clusters precedes rhombomere formation and becomes progressively restricted (43) such that expression boundaries coincide with the interfaces between rhombomeres (44). Their expression patterns form an ordered and nested set of domains along the neuraxis, with a two-rhombomere periodicity. Superimposed on this pattern are rhombomere-specific variations in expression levels (Fig. 4).

Considering the distribution of transcripts and the general synergy among Hox genes detected in mouse null mutants, it is possible that the identity of individual rhombomeres could be defined by the cooperative action of Hox proteins (42). They may also have singular effects: ectopic expression of *Hoxa-1*, for example, results in

the transformation of r2 to an r4 identity (45, 46). However, loss of *Hoxa-1* function results in the deletion of r5, reduction of r4, and loss of specific neuronal nuclei (42), abnormalities that are not obviously consistent with conferring specific identity on an existing repetitive ground plan; but it remains possible that Hox genes could have dual roles, both in segmentation and segment identification.

Positional values appear to be conferred on rhombomeres by Hox expression, but it is unclear how the Hox genes become activated at appropriate levels of the neuraxis. Candidates for this role include *kreisler*, a b-Zip member of the *c-maf* proto-oncogene family (47) expressed in r5 and r6, and *Krox-20*, a zinc finger gene that is expressed in two stripes in the neural plate that become r3 and r5 (48). In *kr*^{-/-} mouse embryos, the neural tube posterior to the r3/r4 boundary appears unsegmented, a defect that is attributable to the loss of r5 and r6 as identifiable territories (49, 50). Targeted disruption of *Krox-20* results in the elimination of r3 and r5 and the formation of a partially fused r2/r4/r6 territory (51). This phenotype suggests that *Krox-20* may be responsible for generating single-compartment periodicity from cues established by

upstream genes. Absence of the r5 stripe of *Krox-20* expression and the more anterior expression of group 4 Hox genes in *kr*^{-/-} mice is consistent with their regulation by *kreisler*, although direct interaction has yet to be shown. In contrast, *Krox-20* is a direct modulator of the r3/r5 activity of both *Hoxa-2* (52) and *Hoxb-2* (53).

A major gap in our understanding of hindbrain segmentation is the lack of candidate segmentation genes. Despite the conserved role of Hox/HOM genes in specifying segmental identity, the upstream pathway appears not to be conserved. However, segmentation is a generic property of metazoan organization that has evolved many times (54), making it likely that Hox/HOM genes have been coupled independently to segmentation.

Retinoid signaling and AP position. In addition to the putative role of *kreisler* and *Krox-20* in locally regulating Hox expression, RA has strong candidacy as an overall mediator of nested Hox expression, consistent with its posteriorizing effect on CNS regionalization. Excess RA causes both an anterior shift of Hox gene expression and an A-to-P transformation of regional fate (55) that includes the ordered transformation of anterior rhombomeres to a more posterior

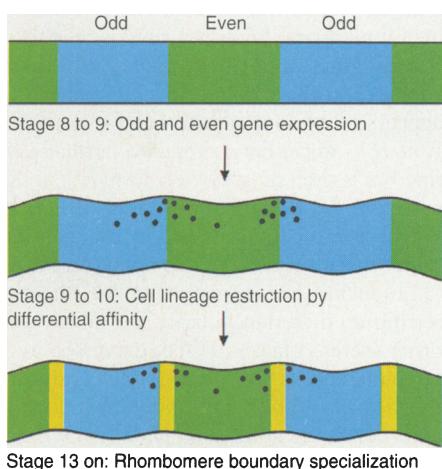


Fig. 3. Stages leading to cell compartmentation and rhombomere boundary (yellow) formation in the chick embryo. An adhesion differential between adjacent domains (blue, green) segregates cells at the interfaces. Although the molecular basis of the differential adhesion is unknown, it follows the same two-segment repeat as displayed by *Krox-20* and other genes shown in Fig. 4. Molecules with boundary-restricted expression (yellow) include *Plzf* (39), *Fgf-3* (40), vimentin (38), low-PSA-NCAM, and laminin (23). Stages numbers refer to normal chick development.

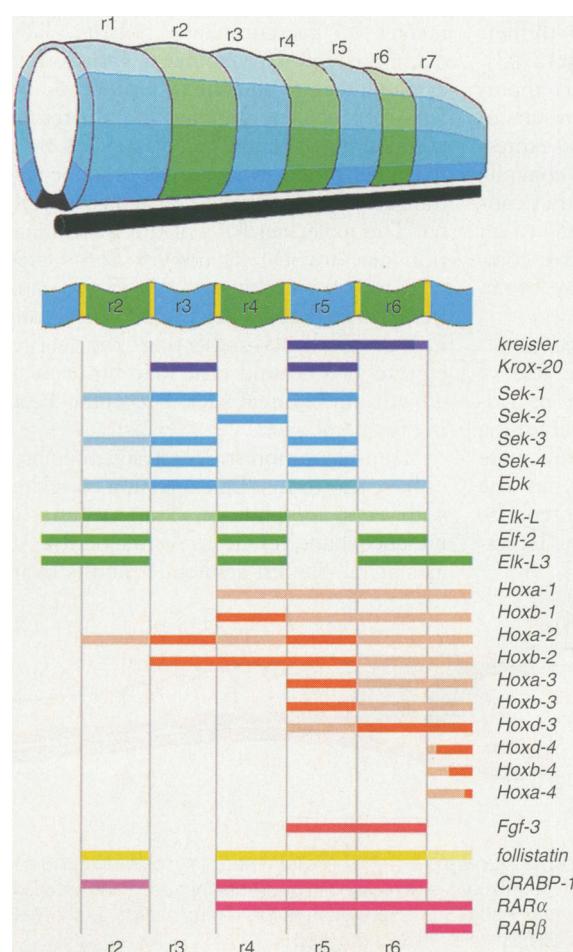


Fig. 4. Summary of the correlation of gene expression with specific rhombomeres, compiled from analysis in mouse and chick embryos. (Top) Odd-numbered rhombomeres are indicated in blue and even-numbered rhombomeres in green, with each segment designated as r1 to r7. The rod-like notochord and overlying floorplate are indicated in black. The vertical yellow lines indicate the boundaries between rhombomeres. The patterns of gene expression are depicted in arbitrary colors with the darkest colors indicating the highest levels of expression. Related genes are indicated by the same color for convenience: Hox homeobox genes (orange), Eph family tyrosine kinase receptors (blue), Eph receptor ligands (green), retinoid- or signaling-related genes (magenta), and early expressed transcription factors (dark purple).

type (56, 57). Conversely, RA-deficient quail embryos have a small hindbrain, lacking posterior rhombomeres (58). In addition, the suppression of RA signaling by expression of a dominant-negative retinoid receptor also results in anteriorization (15). Furthermore, Hox genes have the molecular machinery for responding directly to retinoid signaling (55).

Consistent with a direct role for the organizer, Hensen's node is a rich source of RA and produces increasing amounts during regression (59); thus, the nested expression of Hox genes could be controlled either by a P-to-A gradient of RA diffusing directly from the node, or by an increasing exposure to RA of cells that pass through the node, in A-to-P succession (60). However, it has yet to be shown that RA normally forms a graded signal of either kind or, indeed, that a gradient is necessary; the activity of retinoids could be locally modified by coactivators and corepressors of retinoid signaling (61).

Rhombomere autonomy and plasticity. Transplantation experiments in avian embryos reveal a direct correlation between commitment to rhombomere-specific fate and Hox expression: Grafts of neural plate-stage tissue acquire the complement of Hox transcripts and neuroanatomical features of their new location (62), whereas grafts of emerging rhombomeres maintain both their identity and specific Hox expression (2, 63). By contrast, transplantation of rhombomeres into the post-otic region results in the activation of posterior Hox gene expression (64), suggesting that their commitment is not irreversible. However, they cannot easily be shifted from an even- to an odd-numbered fate, suggesting that commitment to "odd" or "even" may be an early step in segmentation.

In addition, the even-numbered rhombomeres appear to influence the fate of odd-numbered rhombomeres, thus providing a secondary mechanism for establishing positional differences. Inter-rhombomere interactions control cell survival in the neural crest of r3 and r5, the maintenance of Krox-20 expression in r3, and the repression of follistatin in r3 (65).

Fig. 5. Early midbrain patterning. In an early neural tube stage embryo, *Fgf-8* (green) is expressed in a ring of cells at the isthmus, the constriction between the mesencephalic vesicle (M), and rhombomere 1 (r1). *Wnt-1* (yellow) is expressed in a ring of cells immediately rostral to *Fgf-8* and along the dorsal midline. Both *En-1* and *En-2* (blue) are expressed in gradients that decrease anteriorly and posteriorly from the isthmus. Sonic hedgehog (*Shh*) expression, at the ventral midline, is shown in red. T, telencephalon; D, diencephalon; SC, spinal cord; N, notochord.

The diagram illustrates the early neural tube stage of an embryo. It shows the telencephalon (T) at the anterior end, followed by the diencephalon (D) and mesencephalic vesicle (M). The rhombomeric region is labeled r1. The spinal cord (SC) and notochord (N) are at the posterior end. A red arrow points ventrally at the ventral midline. Colored regions indicate gene expression: yellow for Wnt-1 along the dorsal midline, green for Fgf-8 in a ring at the isthmus, blue for En-1 and En-2 in gradients decreasing from the isthmus, and red for Shh at the ventral midline. Dashed lines indicate boundaries between different vesicles.

Midbrain—the Role of the Isthmic Signaling Region

In the midbrain, beyond the anterior limit of Hox gene expression, local AP pattern is generated within an unsegmented field through the activity of a long-range signaling region, the isthmic constriction at the junction of mesencephalic and rhombencephalic vesicles (Fig. 5).

Establishment of midbrain polarity by Engrailed. Signals from the isthmus regulate expression of two *Engrailed* genes (66) in a gradient that decreases both anteriorly, through the mesencephalic vesicle, and posteriorly, through r1 (Fig. 5). Knockout experiments have shown that *En-1* has a critical role in the early specification of the entire region of its expression, whereas *En-2* function is restricted to cerebellar morphogenesis (66). However, the *En-1* mutant phenotype, agenesis of the tectum (dorsal midbrain) and cerebellum (anterior hindbrain), is completely rescued by insertion of the *En-2* complementary DNA into the *En-1* locus (66, 67), demonstrating that the contrasting phenotypes of *En-1* and *En-2* mutations reflect differences in the temporal and spatial expression of the respective proteins and not a divergence in their biochemical activity.

En expression is the earliest known marker for mesencephalic polarity, later manifested in a pronounced variation in cytoarchitecture and the acquisition of different sets of afferent inputs from the retina: the posterior tectum receives axons from the nasal retina, whereas the anterior tectum becomes innervated by temporal retina. The molecular basis of this discrimination may involve ligands for Eph-related receptor tyrosine kinases, RAGS (68) and ELF-1 (69), that are expressed in decreasing P-to-A gradients—reflecting the earlier pattern of *En*—and that may function as growth inhibitors of Mek-4 receptor-bearing temporal axons.

Transplantation studies in avian embryos have shown that *En* expression correlates with later morphology. Thus, when the mesencephalic vesicle is reversed on the AP axis at E2, the *En* gradient readjusts to its

original polarity, and both the graded cytoarchitecture and pattern of retinotectal projections develop normally (66). When reversed at E3, however, the *En* gradient does not adjust, and both cytoarchitecture and retinotectal projection are subsequently inverted. This association has been strengthened by experiments in which *En* is misexpressed in the anterior tectum through use of a retroviral vector: nasal axons arborize ectopically in the anterior tectum, whereas temporal retinal axons fail even to enter the midbrain (70). Furthermore, the altered retinotectal specificity after *En* misexpression in the anterior midbrain is associated with ectopic up-regulation of RAGS and ELF-1, defeating their normal P-to-A expression gradient and effectively converting temporal axon-specific anterior tectum into nasal axon-specific posterior tectum (71). Expression of these effector genes, downstream of *En*, suggests that the normal graded expression of *En* may polarize the dorsal mesencephalon.

Regulation of Engrailed expression. Graded mesencephalic expression of *En* appears to be regulated by signaling from the posterior border of the mesencephalic field. When grafted to the caudal forebrain, the posterior border (isthmus) induces *En* expression and the formation of a complete optic tectum from the surrounding tissue (72). Two secreted signal molecules, *Wnt-1* and *FGF8*, have been implicated in the isthmic control of *En* expression. *Wnt-1*, a homolog of the segment polarity gene *wingless* (a regulator of *Engrailed* in *Drosophila*), is expressed in the midbrain region of the neural plate and later in a ring of cells that lies just anterior to the isthmus. As for their cognates in flies, *Wnt-1* and *En* expression appears to be mutually interdependent: in *Wnt-1*^{-/-} mice, *En* is expressed normally at first but is then progressively lost (73) along with the dorsal midbrain. Thus, although *Wnt-1* is critically involved in the maintenance of *En* expression, it is not a candidate for inducing *En* expression or for directly setting up midbrain polarity. However, another secreted factor, *FGF8*, expressed in a circumferential ring immediately posterior to that of *Wnt-1*, has midbrain-inducing and -polarizing abilities (74). When a bead coated with recombinant *FGF8* is implanted in the posterior diencephalon of chick embryos, expression of *Fgf8*, *Wnt-1*, and *En-2* is induced in the surrounding cells. These cells later display the character of a complete ectopic midbrain, whose AP polarity is reversed with respect to that of the "host" midbrain. Thus, neuroectodermal *Fgf8* expression may be sufficient to establish both midbrain pattern and polarity. *Fgf8* is expressed earlier in axial mesoderm cells that lie beneath the presumptive isth-



mic region of the neural plate (75, 76) and that have the capacity to induce *En* expression (77, 78); mesodermal FGF8 is thus implicated as a homeogenetic inducer in this local control of neural pattern.

Other likely targets of FGF8 are the paired box genes, *Pax-2*, *Pax-5*, and *Pax-8*, which may be required, singly or together, for specification of the isthmus. In *Pax-5*^{-/-} mice (79) and zebrafish treated with function-blocking antibodies to pax(zf[b]), a presumed homolog of Pax-5 (80), the isthmus is deleted. In the zebrafish experiments, the expression of both *Wnt-1* and *En-2* was also repressed, suggesting their direct positive regulation by pax(zf[b]). Indeed, consensus Pax-binding sites have been identified within an enhancer region of the *En-2* gene: when these sites are mutated, the midbrain/hindbrain domain of reporter expression is lost (81).

Whereas isthmic grafts induce tectal development in the caudal diencephalon, the same grafts to the dorsal hindbrain induce cerebellar development (82), demonstrating that the competence of rhombencephalic tissue to respond to isthmic signals differs from that of mesencephalic and caudal diencephalic regions. FGF8 alone appears to be insufficient for inducing ectopic *En-2* expression or cerebellar development in the hindbrain (74), implicating additional signaling molecules at the isthmus.

Although signals from the isthmus are involved in patterning both the dorsal mesencephalon and the dorsal anterior rhombencephalon, the constriction does not correspond precisely with the midbrain/hindbrain junction. Separating structurally and functionally distinct tectal and isthmocerebellar regions of the brain, this junction forms some distance anterior to the constriction and registers with the posterior limit of *Otx-2* expression in the early mesencephalic vesicle (83). The posterior-most, *Otx-2*-negative region of the vesicle is fated to join r1 and r2 in the formation of the cerebellum (84). Thus, it cannot be assumed that obvious morphological features of the neural tube, such as the constrictions between vesicles, necessarily correspond in a predictable way to future subdivisions of the brain.

Forebrain—Is Segmentation Involved?

In contrast to hindbrain and midbrain patterning, where restricted patterns of gene expression have been tightly linked either to segmentation or to the activity of a signaling region, our understanding of early forebrain patterning is virtually limited to the gene expression patterns. Most notable among these (85) are the *Emx*, *Dlx*, and

Nkx homeobox genes, the paired box gene *Pax-6*, the winged helix genes *BF-1* and *BF-2*, the *Brachyury* homolog *Tbr-1*, and the secreted factor-encoding gene *Wnt-3*. Some of these genes are expressed in the ventricular zone, suggesting a role in regional specification, whereas the expression of others (*Dlx* and *Tbr-1*) is restricted to the mantle zone, suggesting a role in the control of differentiation. In the former category, *Emx* and *Otx* genes are expressed in the forebrain and midbrain in a nested array reminiscent of that of the *Hox* genes more posteriorly, although with reversed AP symmetry (86).

Largely on the basis of descriptive molecular studies, it has been proposed that the forebrain is built piecemeal, like the hindbrain, from a series of metamerized units or prosomeres (87). Experimental evidence for compartmentation is limited to the diencephalon where cell lineage restriction boundaries, aligned with prominent axon tracts, define four neuromeres (88). However, the significance of diencephalic neuromeres is brought into question by an analysis of retrovirally marked clones (89), which has shown that sibling cells can occupy multiple nuclei throughout the AP extent of the diencephalon. Further anteriorly, in the telencephalon, the patchwork expression of putative developmental control genes displays no evidence of repetition, the essence of metamerism. Nor does the early cellular organization of the telencephalon support the notion of a segmental origin; rather, this region appears to be subdivided longitudinally into two subregions, the anlagen of cortex (pallium) and striatum (90). These subregions express different regulatory genes (*Emx-1/2*, *Pax-6*, and *Tbr-1* dorsally; *Dlx-1/2* ventrally) and appear to be segregated by differential adhesion (91). Within the dorsal (cortical) subregion, cells migrate extensively in the AP direction, so that clones cross functional boundaries and sibling cells contribute to widely separated structures (92). Supporting the view that the telencephalon is a single field, which becomes subdivided longitudinally, *BF-1* is expressed in the prospective telencephalic domain before the telencephalic/diencephalic boundary appears. In *BF-1*^{-/-} mice, the cerebral hemispheres are severely diminished and ventral telencephalic markers are not expressed (93).

Cell marking and transplantation experiments are required to test the postulate that segmentation is involved in forebrain regionalization. Alternatively, or additionally, forebrain pattern could depend on an as yet undiscovered signaling region. Whether or not a segmented transverse organization exists, a major constraint on understanding forebrain pattern has been our uncertainty

regarding its topological coordinates, particularly with respect to the trajectory of the longitudinal axis. Here, however, detailed descriptions of DV-restricted gene expression patterns (94) have more precisely defined the topology of the domains whose mechanism of formation we are seeking. The rapid accumulation of molecular data has provoked excited speculation: with the combined application of experimental embryological and genetic methods, we can expect this excitement soon to be relieved by enlightenment.

Spinal Cord—Late Role of the Mesoderm in Regionalization

Although superficially uniform, there are subtle variations in cellular composition along the AP axis of the spinal cord. Motor neurons are arranged in discontinuous longitudinal columns that occupy different DV and mediolateral positions at different, plurisegmental levels of the neuraxis. Thus, the neurons that form the lateral motor columns at limb (brachial and lumbar) levels are distinct from those that form at cervical and thoracic levels, not only in the identity of their peripheral targets but also in the expression of different combinations of LIM-homeobox genes that are thought to confer targeting specificity (3, 95). Recent studies of the spinal cord have focused on the control of its pronounced DV pattern (3), whereas classical studies of AP regionalization and the influence of paraxial mesoderm (96) still need to be put into a molecular context. However, genes that lie 5' in the *Hox* clusters have sharp boundaries of expression along the spinal neural tube, suggesting, by analogy with the hindbrain, that they might underlie this regional diversity. Transposition of prospective brachial and thoracic regions leads to the re-specification of *Hox* and *Lim* gene expression, and motor neuron subtypes develop according to their new positions (3). The most likely source of signals that effect the acquisition of this regional identity is the paraxial mesoderm (64). Mesodermal control has also been implicated in the specification of primary motor neurons of the zebrafish: transplantation of single cells to new AP positions with respect to the adjacent somite results in respecification of both the *Lim* gene code of the motor neuron and its subsequent axon trajectory and target specificity (3).

Many aspects of cell pattern are conserved between the hindbrain and the spinal cord, particularly with regard to the DV axis, where ventral (Sonic Hedgehog) and dorsal (BMP) signaling systems appear to be identical in the two regions (3). It is also apparent that these DV signals act on cells

that have already acquired a stable and heritable indication of their position on the AP axis, and thereby an AP position-specific competence to respond (2). The time at which AP fate becomes restricted differs, however, between spinal cord and hindbrain. Whereas the regional AP identity of the spinal neural tube appears to be uncommitted for some time after closure (3, 97), hindbrain pattern is fixed and independent of position relative to the cranial mesoderm from as soon as the rhombomeres become defined (2). The difference may stem from a phylogenetically ancient distinction between head and body with respect to patterning strategy. In the head, the paraxial mesoderm is patently unsegmented and may contribute little to patterning (98), whereas the neural crest predominates, furnishing the ectomesenchymal cells that construct the segmented branchial skeleton and pattern the cranial nerves and muscles. Thus, in the hindbrain/branchial region, segmentally restricted positional information originates in the neural tube and is imposed on the surrounding mesoderm. A relatively rigid set of positional values within the hindbrain region may have evolved both for correct deployment of its emigrant neural crest cells and in compensation for the lack of patterning information in the mesoderm. In the body, by contrast, the mesoderm imposes its AP positional information on the neural tube (99). This is seen both for cell pattern within the tube (3, 100) and for the pattern of motor roots and dorsal root ganglia, whose overtly segmented disposition is controlled, apparently exclusively, by the AP polarity of the somitic sclerotome (101).

Conclusions

Considerable advances have recently been made toward understanding the mechanisms involved in neuraxial regionalization, particularly with respect to the earliest events, during gastrulation, when the molecular identity of activating and transforming signals is being revealed. Especially promising is the evidence, from dominant gain- and loss-of-function experiments with retinoid receptors (15), that RA acts as a concentration-dependent posteriorizing signal *in vivo* and is required for the correct spatial restriction of anterior markers. The action of RA in regionalizing the entire posterior CNS, as studied in early *Xenopus* embryos, is mediated, at least in part, by its direct action on the spatial regulation of *Hox* genes, best known from the amniote hindbrain. A synthesis of data from these diverse experimental systems is needed to advance our understanding of this crucial molecule, as are experiments directed at elucidating its regulation and mode of action, whether as a

gradient from a single source, the organizer, or as discrete local signals from axial or paraxial mesoderm (or both).

On a wider level, expression studies and functional analyses of developmental control genes in different vertebrate systems have revealed the existence of a neuraxial ground pattern that is highly conserved. It will be important to discover the genetic and cellular mechanisms involved in the subsequent elaboration of this ground pattern that produce the very different brains of fish and mammals. Further understanding of CNS regionalization will depend on discovering how region-specifying genes confer a particular potential, or set of potentials, with respect to the ultimate selection of regionally appropriate cell identity.

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Diversity and Pattern in the Developing Spinal Cord

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The generation of distinct neuronal cell types in appropriate numbers and at precise positions underlies the assembly of neural circuits that encode animal behavior. Despite the complexity of the vertebrate central nervous system, advances have been made in defining the principles that control the diversification and patterning of its component cells. A combination of molecular genetic, biochemical, and embryological assays has begun to reveal the identity and mechanism of action of molecules that induce and pattern neural tissue and the role of transcription factors in establishing generic and specific neuronal fates. Some of these advances are discussed here, focusing on the spinal cord as a model system for analyzing the molecular control of central nervous system development in vertebrates.

All neural functions—from simple sensory responses and motor commands to elaborate cognitive behaviors—depend on the assembly of neuronal circuits, a process initiated during embryonic development. An early and fundamental step in this process is the generation of distinct classes of neurons at precise locations within a primitive neural epithelium. Over the past decade, many of the mechanisms that control the identity of specific neural cell types have been defined, in large part through the application of molecular genetics in invertebrate organisms such as *Drosophila* and *Caenorhabditis elegans* but also through cellular and biochemical approaches in vertebrates. Collectively, the study of these diverse systems has provided considerable insight into the relative contributions of environmental signaling and lineage restrictions in neural development and has revealed the identity of many of the extracellular signaling factors and intracellular proteins that direct cell fate.

Some of the most intriguing behaviors depend on the circuits that are formed during the development of the vertebrate brain and spinal cord, yet our understanding of neural development is more fragmentary in the vertebrate central nervous system

(CNS) than in other systems (1). Here we review recent progress in defining how diverse cell types in the vertebrate CNS are generated, focusing largely on the spinal cord, because it is the simplest and most conserved region of the vertebrate CNS (Fig. 1A). In addition, physiological and anatomical analyses of neuronal circuitry in the spinal cord have provided, from the time of Sherrington, a solid cellular framework for interpreting the neural bases of sensory and motor functions (2). Although the functions encoded in spinal cord circuitry are limited by comparison to those of many other brain structures, studies on the development of spinal neurons may reveal general strategies used to establish neuronal diversity and circuitry in more complex regions of the CNS.

We examine the steps involved in the generation of distinct neural cell types through the use of somewhat artificial subdivisions of what is evidently an integrated developmental program.

Induction of the Neural Plate

The development of the spinal cord, as in other regions of the CNS, is initiated by the induction of the neural plate. The classical grafting experiments of Spemann and Mangold in amphibian embryos (3) established that the formation of neural tissue depends on signals provided by prospective

axial mesodermal cells in the organizer region. Until recently the identity and mechanism of action of these endogenous neural inducing factors have remained obscure. Studies of neural induction in *Xenopus* embryos now suggest that in one major pathway of neural induction, factors antagonize the signals mediated by the transforming growth factor- β (TGF β)-like protein, bone morphogenic protein4 (BMP4), which represses neural and promotes epidermal cell fate (4) (Fig. 2).

BMP signaling and neural induction. The possibility that neural induction might result from the inactivation of a signaling pathway that represses neural fate emerged from the observation that dissociation of blastula-stage ectoderm into single cells, presumably preventing intercellular signaling, was sufficient to elicit the formation of neural tissue (5). Members of the TGF β family were suggested to mediate this repressive signal on the basis of experiments designed initially to test whether the TGF β -like protein activin was required for the induction of mesoderm (6). Injection of transcripts that encoded a dominant negative form of an activin receptor blocked mesodermal differentiation. But ectodermal cells expressing this receptor isoform unexpectedly differentiated into neural tissue, suggesting that the blockade of activin receptor signal transduction is sufficient to trigger neural induction. Two lines of evidence indicate that BMP4 rather than activin itself is likely to be the endogenous TGF β -like protein that interacts with this receptor and represses neural differentiation. First, BMP4 is widely expressed in the early ectoderm and its expression is extinguished from neural plate cells during neural induction (7). Second, BMP4 but not activin can prevent the expression of neural markers and promote epidermal differentiation in dissociated ectodermal cells (8). Organizer-derived signals might therefore induce neural tissue by means of endogenous proteins that block signaling mediated by BMP proteins.

Support for this idea has come from the demonstration that three candidate neural inducers expressed by organizer tissue can act in this manner (Fig. 2, B and C). The endogenous activin-binding protein follistatin is expressed by organizer cells, and

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