4. ECTODERM: NEURULATION, NEURAL TUBE, NEURAL CREST

Dr. Taube P. Rothman
P&S 12-520
Tpr2@columbia.edu
212-305-7930

Recommended Reading: Larsen Human Embryology, 3rd Edition, pp. 85-102, 126-130

Summary:

In this lecture, we will first consider the induction of the neural plate and the formation of the neural tube, the rudiment of the central nervous system (CNS). The anterior portion of the neural tube gives rise to the brain, the more caudal portion gives rise to the spinal cord. We will see how the requisite numbers of neural progenitors are generated in the CNS and when these cells become post mitotic. The molecular signals required for their survival and further development will also be discussed. We will then turn our attention to the neural crest, a transient structure that develops at the site where the neural tube and future epidermis meet. After delaminating from the neuraxis, the crest cells migrate via specific pathways to distant targets in an embryo where they express appropriate target-related phenotypes. The progressive restriction of the developmental potential of crest-derived cells will then be considered. Additional topics include formation of the fundamental subdivisions of the CNS and PNS, as well as molecular factors that regulate neural induction and regional distinctions in the nervous system.

Learning Objectives:

At the conclusion of the lecture you should be able to:

1. Discuss the tissue, cellular, and molecular basis for neural induction and neural tube formation. Be able to provide some examples of neural tube defects caused by perturbation of neural tube closure.

2. Explain how neuronal precursors are generated in the CNS.

3. Describe the early changes in neural tube shape and the formation of the primary brain vesicles.

4. Discuss the ways in which two important signaling molecules, Sonic hedgehog (Shh) and bone morphogenic protein (BMP-4), regulate expression of regional distinctions in the nervous system.

5. Discuss where and how the neural crest forms, the origin of the migratory pathways that lead crest-derived cells to specific targets, and the effect of the genetic and environmental cues they encounter as they migrate and differentiate.

Glossary of Terms:

Anencephaly: failure of the anterior neural tube region to close.

Cavitation: formation of a space within a mass of cells.

Delaminate: cells dissociate from an embryonic epithelial layer and migrate as mesenchymal cells.

Differentiation: expression of a given cellular phenotype.

Ectopic: outside the normal position; e.g., transplantation of an embryological structure to a new (ectopic) site.

Floor plate: specialized non-neuronal cells situated at the ventral midline of the neural plate/tube.

Heterotopic transplantation: see ectopic

Neural crest: a transient structure composed of cells originally located in the dorsal most portion of the neural folds and closing neural tube.

Neural folds: bilateral elevated lateral portions of the neural plate flanking either side of the neural groove.

Neural groove: a midline ventral depression in the neural plate.

Neural plate: that portion of the dorsal ectoderm that becomes specified to become neural ectoderm.

Neuraxis: the brain and spinal cord. In developmental terms the term refers to the neural tube, from its rostral to caudal end.

Neuroblast: an immature neuron.

Neuroepithelium: a single layer of rapidly dividing neural stem cells situated adjacent to the lumen of the neural tube (ventricular zone).

Neuropore: open portions of the neural tube. The unclosed cephalic and caudal parts of the neural tube are called anterior (cranial) and posterior (caudal) neuropores, respectively.

Neurotrophic factors: proteins released from potential targets that promote or inhibit neuronal survival.

Neurulation: the process by which neural plate develops into a neural tube.

Roof plate: analogous to floor plate but on the dorsal surface of the neural tube.

Primary neurulation: development of the neural tube from neural plate.

Secondary neurulation: development of the neural tube from mesenchyme caudal to the posterior neuropore (tail bud).

Sonic hedgehog (Shh): secreted paracrine factor that induces specific transcription factors. Made by notochord and floor plate.

Spina bifida: a birth defect resulting from an unclosed portion of the posterior neural tube or subsequent rupture of the posterior neuropore soon thereafter.

Transcription factors: activate genes encoding proteins.

Text:

The epidermis, the central and peripheral nervous systems, and some non-neuronal cells of the head and heart are derived from ectoderm (Figure 4-1). During the third week of gestation a portion of the dorsal ectoderm is specified to become neural ectoderm. This region of the embryo is called the neural plate. The process by which the neural plate forms a **neural tube** is called **neurulation**.

I. Primary neurulation: This term refers to the formation of the neural tube from the neural plate, situated between the anterior and posterior neuropores (see figure 4-6).

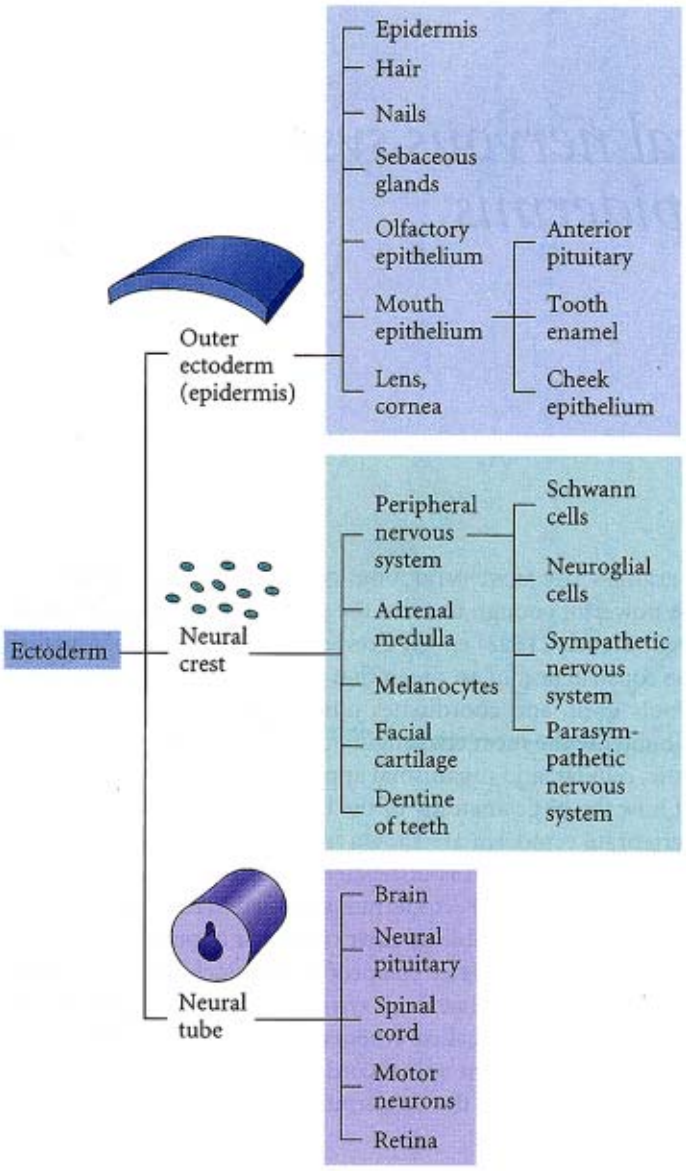

Fig. 4-1. Major derivatives of the ectodermal germ layer. The ectoderm is divided into three major domains the surface ectoderm (primarily epidermis), the neural tube (brain and spinal cord), and the neural crest (peripheral neurons, pigment, facial cartilage).(Gilbert, Developmental Biology, 6th edition)

At the tissue level, neurulation occurs in four stages (Figure 4-2): (i) transformation of the central portion of the embryonic ectoderm into a thickened neural plate (ii) shaping and elongation of the neural plate, (iii) bending of the neural plate around a medial groove followed by elevation of the lateral folds (iv) closure of the neural tube. Note that the term "neurulation" specifically refers to stage (iii) but the name is commonly used when describing all of the events that occur between neural induction and neural tube closure.

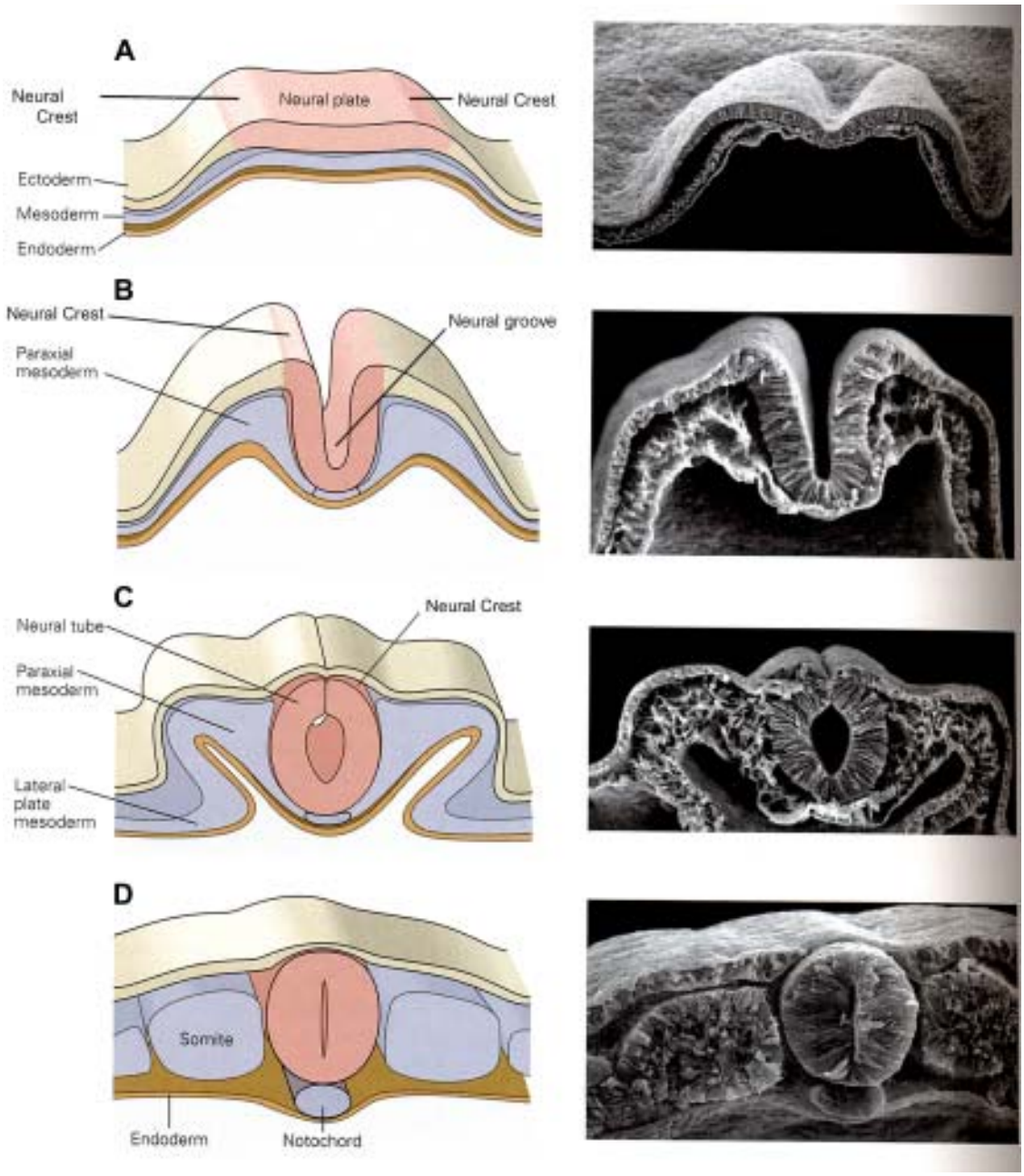

Fig. 4-2. The neural plate folds in stages to form the neural tube. (Scanning electron micrographs of chick embryos provided by G. Schoenwolf.) A. Position of the neural plate in relation to the nonneural ectoderm, the mesoderm, and the endoderm. B. Folding of the neural plate to form the neural groove. C. Dorsal closure of the neural folds to form the neural tube and neural crest. D**.** Maturation of the neural tube and its position relative to the axial mesodermal structure, notochord, and somites (derived from the paraxial mesoderm). (Adapted from Jessell & Sanes, Principles of Neuroscience 4th edition, 2002, E. Kandel editor)

1. Neural induction-formation of the neural plate

Neural induction is the first step whereby the uncommitted or naïve ectoderm becomes committed to the neural lineage. During gastrulation, signals from the node or its derivative, the notochord, induce commitment. Classical studies led to the notion that inducing substances, secreted by the underlying

prechordal plate and the cranial portion of the notochordal plate, were responsible for ectodermal commitment to a neuronal lineage by the overlying epiblast cells. There is now good evidence that 'neural induction' actually involves suppression of induction of an epidermal fate rather than induction of a neural fate so that the default state of the naïve ectoderm is neural, not epidermal as suggested by older studies. In amphibians, molecules (e. g. noggin, chordin, follistatin) that inhibit the expression of bone morphogenetic protein-4 **(BMP-4)** appear to block epidermal expression (Figure 4-3). Although

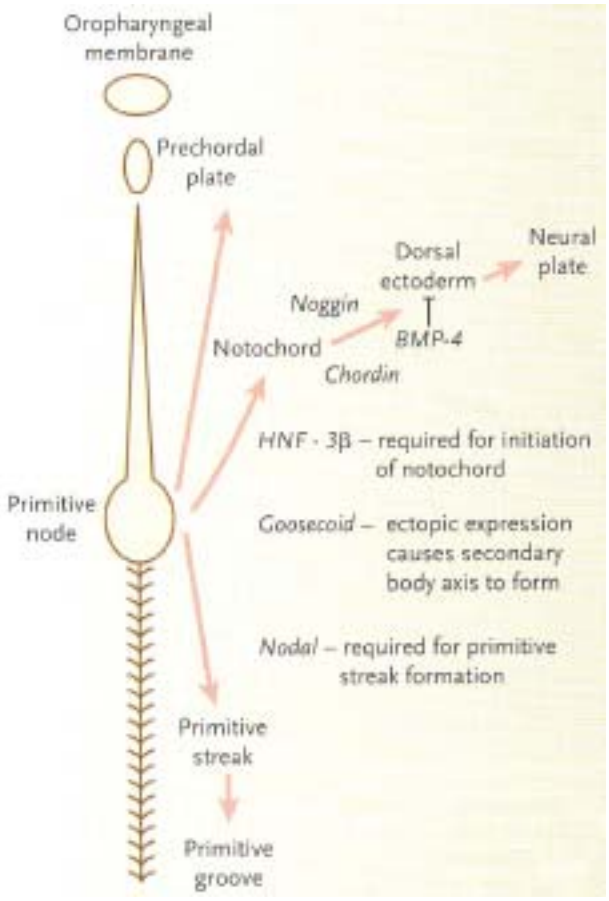

Fig. 4-3 Summary of major genes involved in gastrulation and neural induction. Names of specific genes (*italics*) are placed by the structures in which they are expressed (Carlson, Human Embryology & Developmental Biology, 2nd edition)

the suppression signal has been shown to be generated by Hensen's node in birds, suppression of BMP-4 may not be the only requirement for neural induction in mammals.

The principal early morphological response of the embryonic ectoderm to neural specification is an increase in the height of the cells destined to become components of the nervous system. These transformed cells, now known as **neuroepithelial cells** or **neuroectoderm**, are evident as a thickened **neural plate** visible on the medial dorsal surface of the early embryo (Figures 4-2).

2. Shaping of the Neural Plate: (Figure 4-4; also see Larsen, Figures 3-4 and 3-14). At the time of its formation, the neural plate is shaped like a spade being relatively wide mediolaterally and short rostrocaudally. The caudal wings of the spade flank the primitive node. During shaping, the nascent neural plate becomes narrower and longer. Although the processes of neurulation and gastrulation can be uncoupled experimentally, full craniocaudal formation and extension requires the normal cellular movements of gastrulation.

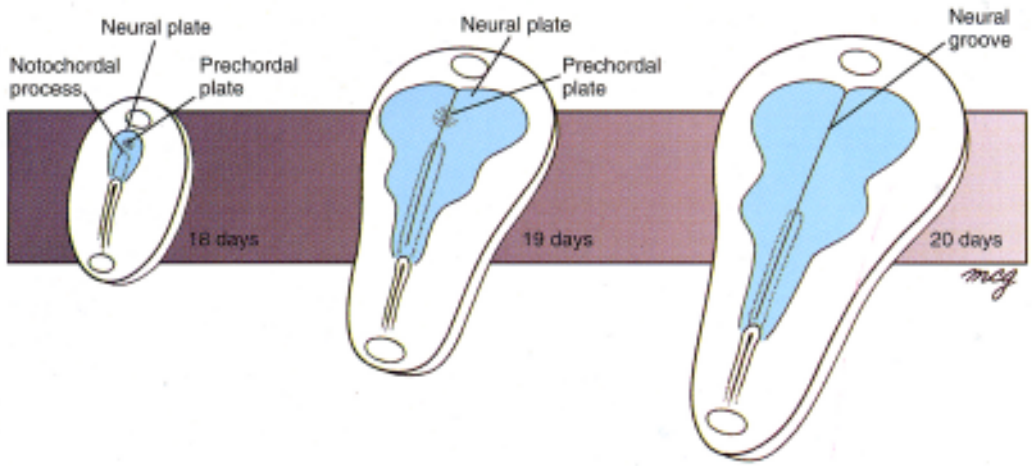

Fig. 4-4. A schematic sequence showing how the neural plate grows and changes proportions between day 18 and day 20. The primitive streak shortens only slightly, but it occupies a progressively smaller proportion of the length of the embryonic disc as the neural plate and embryo grow (Larsen, 3rd edition, Fig.3-13).

3. Neurulation (Figure 4-2): The original ectoderm can be divided into three sets of cells: (i) the internally positioned neural plate, (ii) the externally positioned future epidermis of the skin, (iii) and the neural crest cells that connect the neural plate and epidermis. Lateral folding or bending of the neural plate results in elevation of two walls, **the neural folds**, flanking a ventral midline **floor plate** (composed of non-neuronal cells) of the **neural groove**. **Formation of the neural tube** occurs when the two dorsolateral apical surfaces of the neural folds meet, fuse at the dorsal midline, and separate from the overlying ectoderm. Forces generated by the surface epithelium as it expands towards the dorsal midline cause elevation of the neural folds and ultimately, closure of the neural tube. Bends in the medial portion of each neural fold maintain the structure of the tube so that the lumen remains patent as the neural folds converge.

The molecular signals for primary neurulation in human embryos (Figure 4-5) remain largely unknown but several candidate genes that perturb neurulation when mutated have now been identified. Sonic hedgehog (**Shh**) is an important signaling center. Not only does it induce elevation of neural folds but also the formation of the neural groove and floor plate. In the dorsal portions of the future neural tube, **Wnt6**, secreted by the epidermal ectoderm adjacent to the neural plate and **BMPs** induce **slug** in the future neural crest cells (see section on neural crest below). The BMPs also appear to maintain dorsal expression of **Pax transcription factors**. Shh signaling from the floor plate, suppresses the expression of dorsal Pax genes in the ventral half of the neural tube where motor neurons develop.

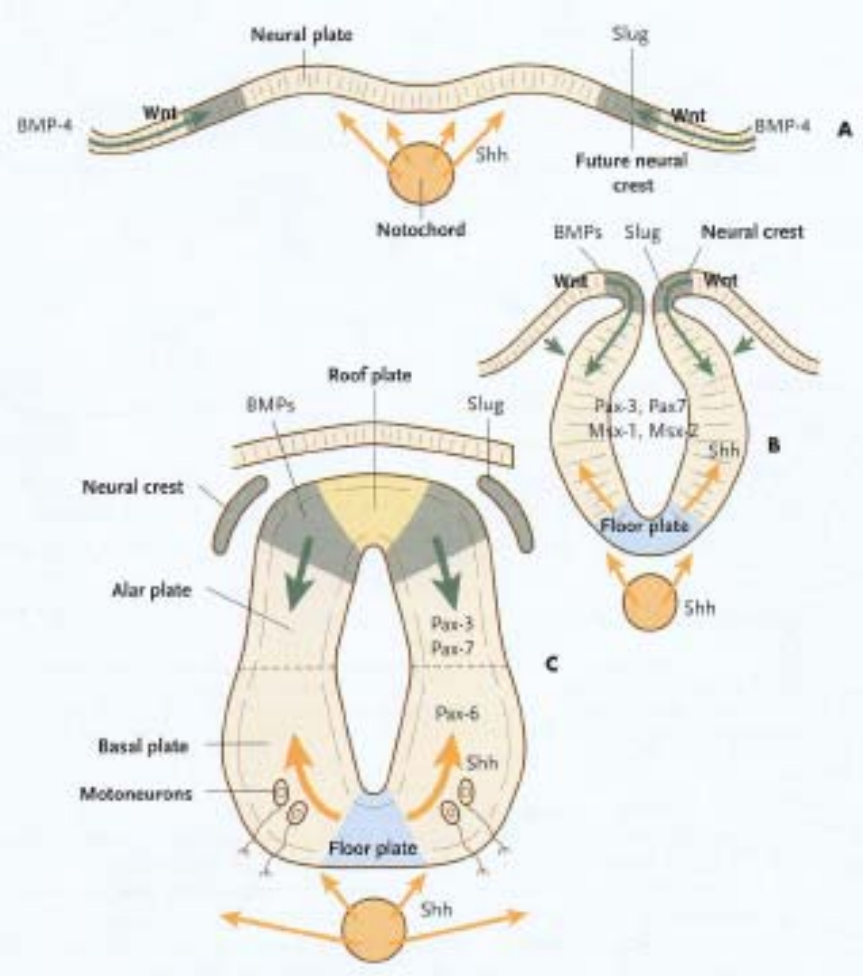

Fig.4-5. Dorsal and ventral signaling in the early central nervous system. **A**, Signals from sonic hedgehog (Shh)(orange arrows)in the notochord induce the floor plate. **B**, In the dorsal part of the future neural tube, Wnt from the ectoderm adjacent to the neural tube induces slug in the future neural crest and maintains Pax-3 and Pax-7 expression dorsally. Ventrally, sonic hedgehog, now produced by the floor plate, induces motoneurons. **C**, Sonic hedgehog, produced by the floor plate, suppresses the expression of dorsal Pax genes (Pax-3 and Pax-7) in the ventral half of the neural tube. (Carlson, Human Embryology & Developmental Biology, 2nd edition)

Closure of the neural tube begins almost midway along the craniocaudal extent of the nervous system of the 21-22 day human embryo (Figures 4-6A,B). Over the next couple of days, closure extends both cephalically and caudally in a manner resembling the closing of a double-headed zipper. The unclosed cephalic and caudal parts of the neural tube are called the **anterior (cranial) and posterior (caudal) neuropores.** The neuropores will ultimately close (24 days gestation for the cranial neuropore and 26 days for the caudal) so that the future central nervous system (CNS) is organized in a way that resembles an irregular cylinder sealed at both ends. Neural tube defects occur when various parts of the neural tube fail to close. An open posterior neuropore results causes **spina bifida** (Figure 4-6E), the severity of which depends on the length and position of the open segment. **Anencephaly** (Figure 4-6D) is a lethal condition in which the anterior neuropore fails to close. The forebrain remains in contact with the amniotic fluid and subsequently degenerates.

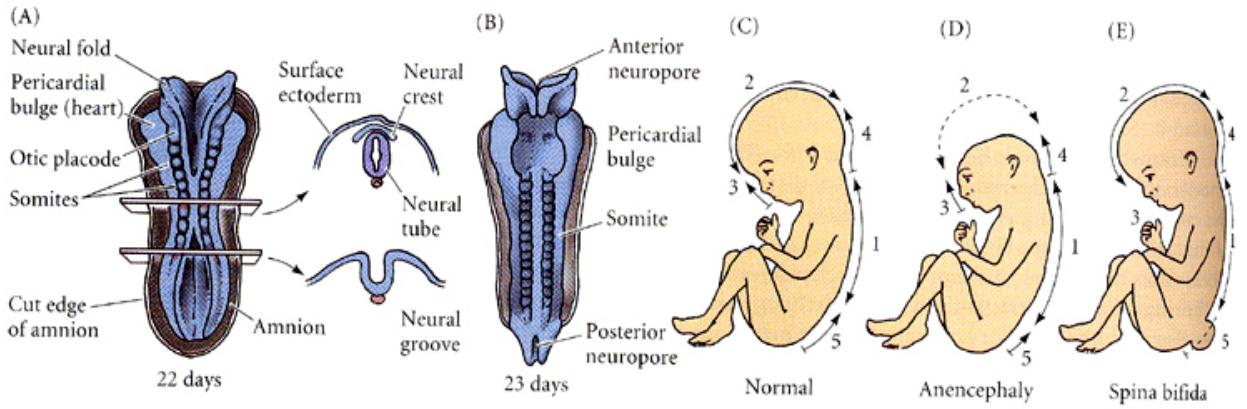

Fig. 4-6. Neurulation in the human embryo. (A)Dorsal and transverse sections of a 22 day human embryo initiating neurulation. Both anterior and posterior neuropores are open to the amniotic fluid. (B)Dorsal view of the neurulating human embryo a day later.The anterior neuropore region is closing while the posterior neuropore remains open.(C)Regions of neural tube closure postulated by genetic evidence (superimposed on newborn body). (D) Anencephaly is caused by the failure of neural plate fusion in region 2. (E) Spina bifida is caused by the failure of region 5 to fuse (or of the posterior neuropore to close).(C-E after Van Allen et al. 1993.)(Gilbert, Developmental Biology, 6th edition)

II. Secondary neurulation:

Caudal to the posterior neuropore, the neural tube is formed by the process of secondary neurulation (Figure 4-7). A rod like condensation of mesenchymal cells forms beneath the dorsal ectoderm of the tail bud. Within the mesenchymal rod, a central canal forms by cavitation. This central canal becomes continuous with the one formed during primary neurulation and closure of the posterior neuropore. Because of the diminished development of the tail bud in humans, secondary neurulation is not a prominent process.

Fig. 4-7. Secondary neurulation in the caudal region of a 25 -somite chick embryo. (From Catala et al. 1995; photographs courtesy of N.M. Le Douarin.)(Gilbert, Developmental Biology, 6th edition)

III. The neural tube forms the primordia of the central nervous system:

Even before the neuropores have closed the future brain and spinal cord are recognizable and the brain becomes subdivided into a forebrain (**prosencephalon**), midbrain (**mesencephalon**) and hindbrain (**rhombencephalon**) (Figure 4-8, 4-9). The increased volume of the early brain is the result of an increase in cavity size, not tissue growth. In the chick embryo, brain volume expands 30 fold between

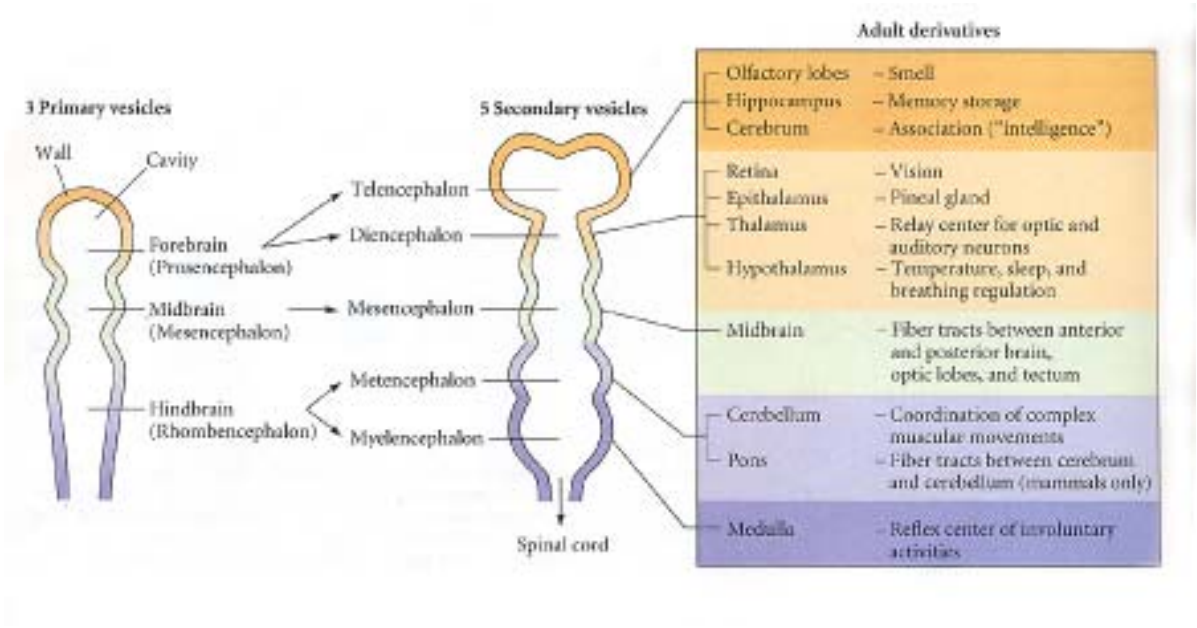

Fig. 4-8. Early human brain development. The three primary brain vesicles are subdividzed as development continues. At the right is a list of the adult derivatives formed by the walls and cavities of the brain.(After Moore and Persaud 1993.) (Developmental Biology, 6th editon, S. Gilbert)

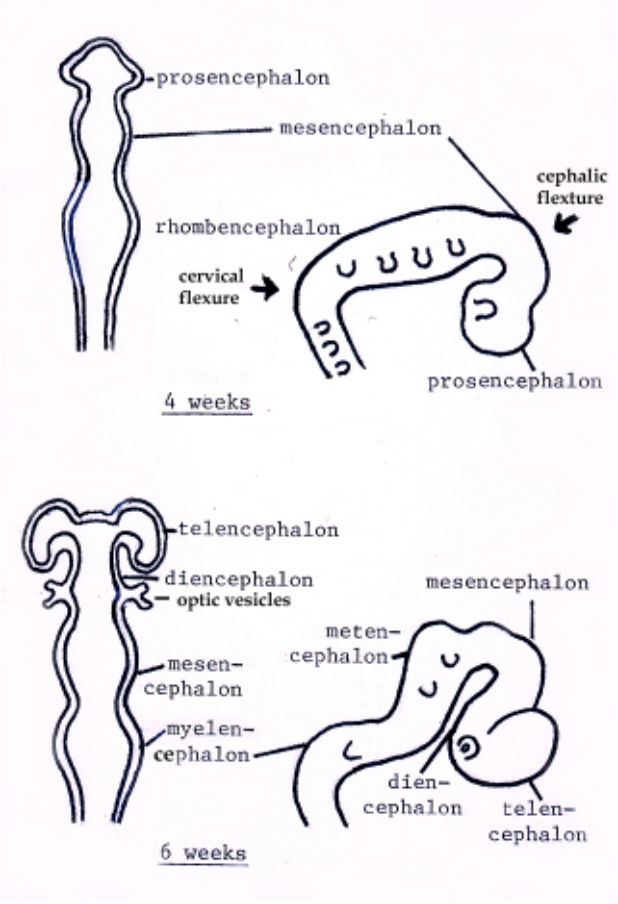

Fig. 4-9. Basic anatomy of the three-part (A) and five-part (B) human brain.

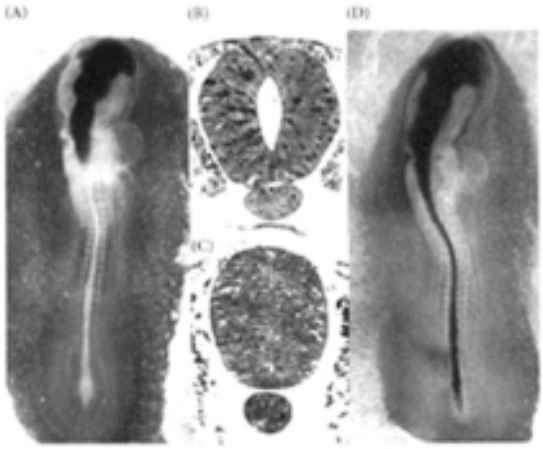

Fig. 4-10. Occlusion of the neural tube allows expansion of the future brain region. (A) Dye injected into the anterior portion of a 3-day chick neural tube fills the brain region,but does not pass into the spinal region. (B,C) Section of the chick neural tube at the base of the brain (B) before occlusion and (C) during occlusion. (D) Reopening of the occlusion after initial brain enlargement allows dye to pass from the brain region into the spinal cord region. (Photographs courtesy of M. Desmond.) (Gilbert, Developmental Biology, 6th editon,)

days 3 and 5 of development. This rapid expansion is thought to be caused by pressure from fluid exerted against the walls of the neural tube after the surrounding dorsal tissues push in to temporarily constrict the neural tube in the region between the presumptive brain and spinal cord (Fig 4-10). The occluded region reopens after the initial rapid enlargement of the brain vesicles. Another prominent force in shaping the early nervous system is the overall bending of the cephalic end of the embryo into a C shape. Associated with this bending is the appearance at the end of the third week of a prominent **cephalic flexure** of the brain at the level of the mesencephalon (Figure 4-9). Soon the brain almost doubles back on itself at the cephalic flexure. At the beginning of the fifth week a second cervical flexure appears at the boundary between the hindbrain and the spinal cord. By the end of the fifth week the prosencephalon becomes further subdivided into a **telencephalon** and a more caudal **diencephalon** with prominent **optic vesicles** extending from its lateral walls (Figures 4-8, 4-9). The rhombencephalon divides into the **metencephalon** and more caudally, the **myelencephalon**. These five the **primary brain vesicles**, plus the spinal cord, comprise the early fundamental organization of the CNS. For purposes of this course you are not required to learn the derivatives of the primary brain vesicles. Future lectures in the Neuroscience course will address such issues as functional development and molecular patterning of the brain and spinal cord.

Fig.4-11. Scanning electron micrograph of a newly formed chick neural tube, showing cells at different stages of their cell cycles (Courtesy of K. Tosney.)Gilbert, Developmental Biology, 6th editon,)

IV. Cell proliferation within the neural tube:

The original neural tube is lined by a **ventricular zone**, composed of a single layer of rapidly dividing neural stem cells, called the **neuroepithelium** (sometimes known as a germinal epithelium). All the cells of the neuroepithelium extend to the luminal surface but their nuclei are at different heights thereby giving the structure a pseudostratified appearance (Figure 4-11). DNA synthesis (S phase) occurs while the nucleus is positioned at the outside edge of the zone (Figure 4-12). As the cell cycle proceeds, the nucleus migrates within the cell cytoplasm toward the lumen. Mitosis occurs at the luminal side of the ventricular zone and the two daughter cells then continue to cycle. A cell that has undergone its last mitotic division is derived from a stem cell that divides parallel to the ventricular surface (Figure 4-12). The daughter cell adjacent to the lumen remains connected to the ventricular surface, continuing in the cell cycle, while the post mitotic daughter migrates out of the germinal epithelium.

V. Neurons are post-mitotic cells:

The time of a neuron's last 'S' (last time cell replicates its DNA) is called the neuron's birthday. Although some neuroblasts can be induced to divide in vitro, neurons do not enter 'S' and do not divide. The birthdays and sequence of origin of neurons and glial cells in the CNS (and the PNS for that matter) can be observed by utilizing thymidine or uridine analogues (bromodeoxyuridine [BrdU], ^{3}H-thymidine)

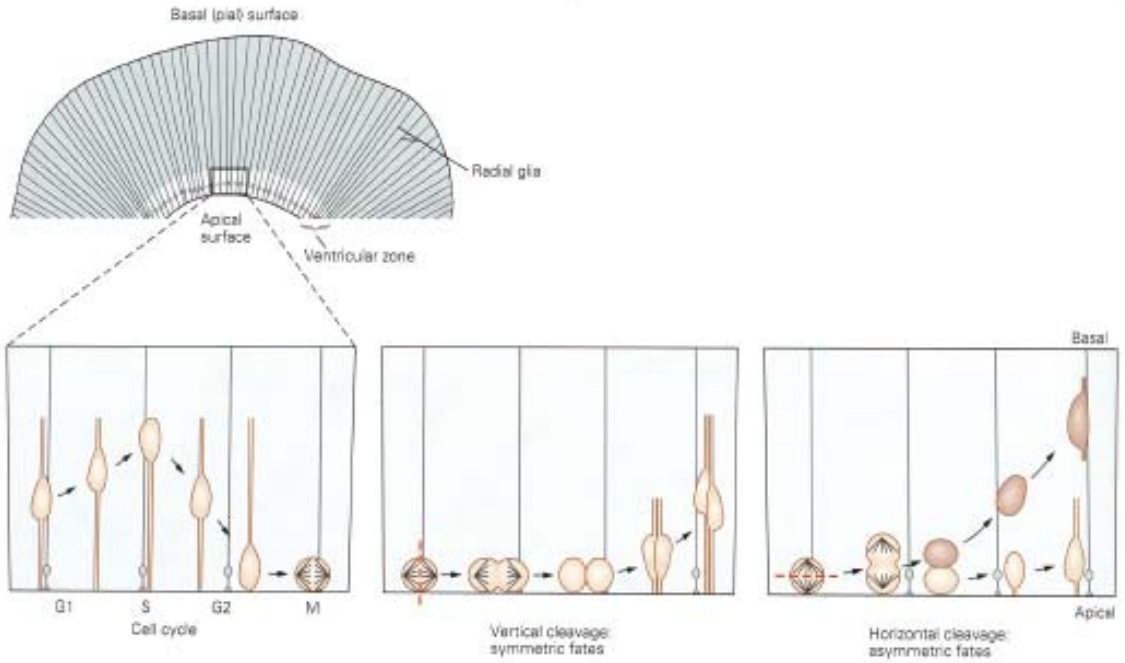

Fig. 4-12. The plane of division of progenitor cells in the ventricular zone of the cerebral cortex influences their fate. The nuclei of ventricular zone precursors migrate during the cell cycle. During the G1 phase of the cell cycle, nuclei rise from the inner (apical) surface of the ventricular zone. During the S phase the nuclei reside in the outer (basal) third of the ventricular zone. During G2 the nuclei migrate apically, and mitosis occurs when the nuclei reach the ventricular surface. Cleavage of progenitor cells perpendicular to the ventricular surface generates two similar daughters that retain their apical connections. Following mitosis, the nuclei of both cells reenter the cell cycle. Cleavage that is parallel to the ventricular surface produces an asymmetric division in which the apical daughter retains contact with the apical surface and the basal daughter loses its apical contact. The basal daughter migrates away from the ventricular zone and later becomes a postmitotic neuron. (Adapted from Chen and McConnell 1995.)(Jessell & Sanes, Principles of Neuroscience 4th edition, 2002, E. Kandel editor)

as markers to recognize the last time a cell undergoes 'S'. Such labeling studies have demonstrated that CNS neurons assemble either by stacking in laminar structures or by packing into nuclear regions. For example, in the cerebral cortex, those neurons that are born first, at early stages, migrate to the deepest layers of the cortex while those born later take up positions successively farther from the ventricular germinal zone from which they originated (Figure 4-13).

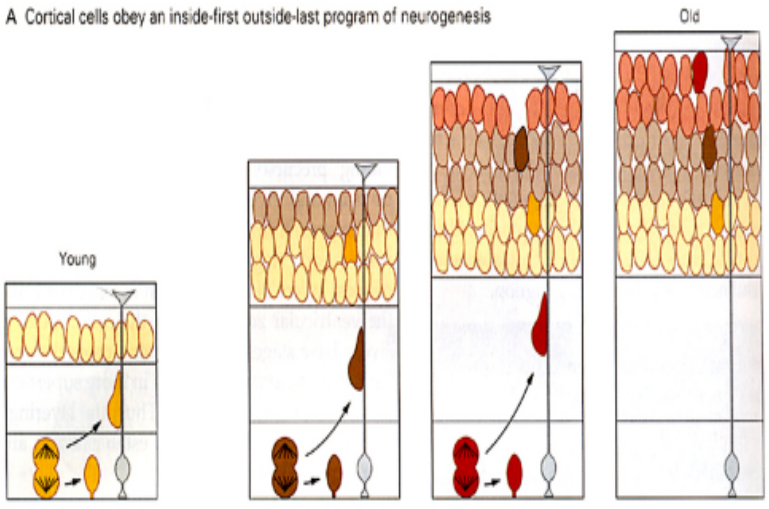

Fig. 4-13. Generation and migration of neurons in the mammalian cerebral cortex. (Adapted from Chenetal. 1997.) Cortical neurons are generated in an inside-first, outside-last order. Neurons born within the ventricular zone at early stages migrate to the deepest layers of the cortical plate. Neurons generated at later stages migrate past the earlier-generated neurons to form the more superficial layers of the cortex. (Jessell & Sanes, Principles of Neuroscience, 3rd edition, 2000, E. Kandel, editor)

VI. Neuronal survival depends upon target related trophic signals:

It should be noted that not all neuroblasts survive. Of the huge number generated, nearly half are destined to undergo apoptosis and die (Figure 4-14). Only those neurons that make structural and functional synaptic connections with specific targets are not eliminated. Both in CNS and PNS, neuronal survival and neuronal cell death are under the tight developmental control of gene products secreted by target structures. These trophic factors are required to sustain growth and survival. Cell death and neuronal survival will be the subject of two subsequent HD lectures 15 and 16.

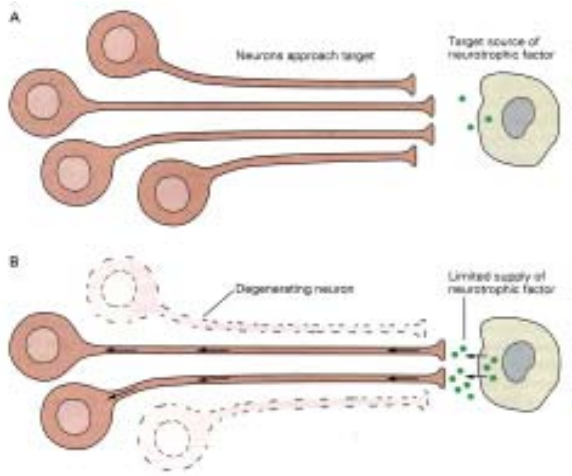

Fig. 4-14. The neurotrophic factor hypothesis. (Adapted from Reichardt and Farinas 1997.) A. Neurons extend axons to the vicinity of target cells. B. The target cells secrete limited amounts of neurotrophic factors. The neurotrophic factors bind to specific cell surface receptors. Neurons that do not receive adequate amounts of neurotrophic factor die by apoptosis.(Jessell & Sanes, Principles of Neuroscience/ 2000, E. Kandel editor)

VII. The Neural Crest:

1. At the time the neural plate becomes specified, an interaction between the surface ectoderm (SE) and neural plate (NP) creates an intermediate structure, known as the neural crest (Figure 4-15, 4-5). (This structure has sometimes been referred to, by those who study it, as the 'fourth germ layer'). During neural tube closure, when the dorsal tips of the neural folds converge, these cells delaminate (recall the motile cells that **delaminate** from the epiblast during gastrulation; Lecture 2) from the dorsal neural tube and migrate from their original sites in the neuraxis, via

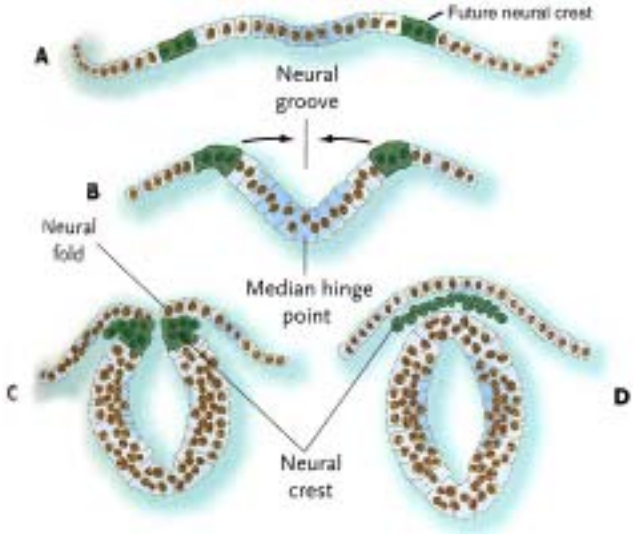

Fig. 4-15. Cross sections through the forming neural tube. A, Neural plate. B, Neural fold. C, Neural folds apposed. D, Neural tube complete. (Neural crest before and after its exit from the neural epithelium is shown in green.)(Human Embryology & Developmental Biology, 2nd editon, Carlson, B.M.)

specific routes, to colonize peripheral targets. Crest-derived cells are capable of differentiating into an astonishing number of different and diversified cell types and tissues including Schwann cells or glial cells of the sensory, sympathetic, parasympathetic, and enteric nervous systems, cells of the adrenal medulla, pigment cells in the epidermis, and connective tissue components of the head (Table 4-1), yet they express only those phenotypes that are appropriate for the organ to which they have migrated.

Derivative	Cell type or structure derived
Peripheral nervous system (PNS)	Neurons, including sensory ganglia, sympathetic and parasympathetic ganglia, and plexuses Neuroglial cells Schwann cells
Endocrine and paraendocrine derivatives	Adrenal medulla Calcitonin-secreting cells Carotid body type I cells
Pigment cells	Epidermal pigment cells
Facial cartilage and bone	Facial and anterior ventral skull cartilage and bones
Connective tissue	Corneal endothelium and stroma Tooth papillae Dermis, smooth muscle, and adipose tissue of skin of head and neck Connective tissue of salivary, lachrymal, thymus, thyroid, and pituitary glands Connective tissue and smooth muscle in arteries of aortic arch origin

Source: After Jacobson 1991, based on multiple sources.

Table 4-1. Some derivatives of the neural crest

2. The inductive action by the non-neural ectoderm on the laterally situated cells of the neural plate, now appears to be mediated by members of both the **Wnt** family of genes, particularly ***Wnt-6*** which has recently been shown to be expressed by the non-neuronal ectoderm and by **BMP**s that are expressed in the neural folds (Figure 4-15). Once induced, neural crest cells express **slug**, a transcription factor that mediates dissociation (delamination) of cells from an embryonic epithelial layer. Slug appears to activate factors that dissociate the tight junctions between crest cells, allowing them to change their shape and properties from those of typical neuroepithelial cells to those of mesenchymal cells (note, that even though the crest cells become mesenchymal, they are of ectodermal origin). In the head, neural crest cells delaminate before the neural folds fuse. In the trunk, they do not leave the neuroepithelium until the neural tube has fully formed. During the epithelial-to-mesenchymal transformation, the migrating neural crest cells discontinue the expression of the cell surface adhesion molecule N-cadherin, but express it again once they aggregate to form the spinal and sympathetic ganglia.

Origin of neural crest migration pathways that lead crest-derived cells to target organs:

Studies utilizing avian chimeric embryos (Le Douarin and colleagues) have provided a great deal of information regarding the specificity of individual neural crest migration pathways, as well as the developmental potential and restriction of crest-derived phenotypes. Neural crest migration routes originate from specific sites along the cranial-caudal axis (neuraxis) of the dorsal neural tube. The

migration pathways lead the dividing crest-derived cells to specific end targets where they stop dividing and differentiate into target related phenotypes. Thus, the site in the neuraxis from which a crest cell originates, determines the target it will reach (Figure 4-16). Heterotopic (ectopic) transplantation of crest cells into a migration pathway that they normally do not traverse, leads them to a new target where, depending on their developmental potential, they may express a new phenotype that is appropriate for the target they have colonized. Research conducted on mammalian embryos suggests that except for relatively minor structural details, information learned from birds can be directly applied to mammalian development.

The crest-derived cells that reach a target at the end of migration pathway are different than those that entered it. As they migrate, they encounter extracellular signaling molecules, e.g. **growth factors** and **trophic factors**, and components of the extra cellular matrix, e. g. **fibronectin, laminin and collagen IV**, that are conducive for their continued migration and proliferation (discussed further by Dr. Greene in HD lecture 16). As they migrate, the crest-derived cells develop appropriate receptors that allow them to interact with these environmental cues so that by the time they reach their specific target, their number has increased significantly.

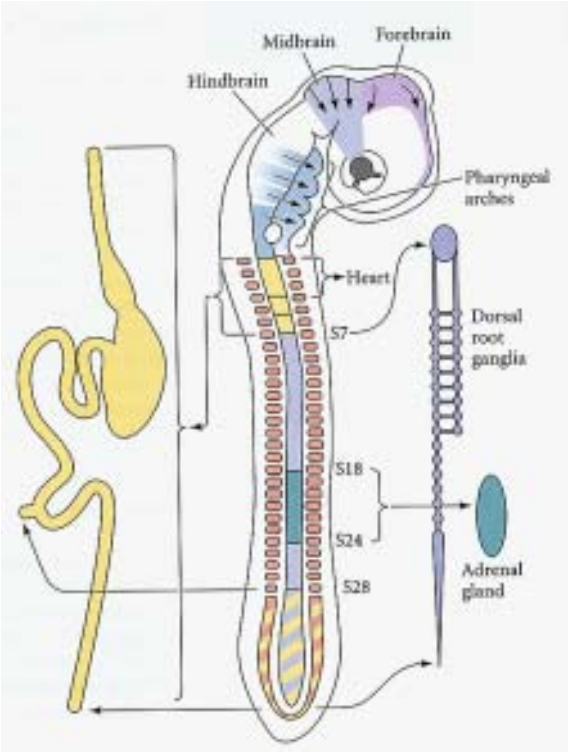

Fig. 4-16. Regions of the neural crest. The cranial neural crest migrates into the branchial arches and the face to form the bones and cartilage of the face and neck. It also produces pigment and cranial nerves. The vagal neural crest (near somites 1-7) and the sacral neural crest (posterior to somite 28) form the intrinsic neurons of the gut. The cardiac neural crest cells arise from the neural crest near somites 1-3; they are critical in making the division between the aorta and the pulmonary artery. Neural crest cells of the trunk (about somite 6 through the tail) make the sympathetic neurons, and a subset of these (at the level of somites 18-24) form the medullary portion of the adrenal gland. (After Le Douarin 1982.) (Developmental Biology, 6th editon, S. Gilbert)

The cranial neural crest: Crest-derived cells in the head region produce the craniofacial mesenchyme that differentiates into cartilage and bone, cranial neurons and glia and connective tissues of the face. Other cells enter pathways traversing pharyngeal structures where they give rise to such diversified cells as those of the thymus, odontoblasts of the tooth primordia and the bones of the middle ear and jaw. The migration pathways followed by cranial neural crest cells will be discussed in more detail at a future time (HD 9).

In the trunk: (adjacent to somites 8-28), crest-derived cells follow one of three pathways. (i) Cells that migrate along the dorsolateral pathway become melanocytes. These cells travel through the dermis entering the ectoderm through minute holes in the basal lamina. Here they colonize the skin and hair follicles. (ii) This pathway takes trunk neural crest cells more ventrolaterally through the **anterior half of each sclerotome** (Figure 4-17 A-D). Sclerotomes will be discussed in the next lecture but briefly, they consist of segmented blocks of mesoderm, derived from somites that differentiate into vertebral cartilage. The anterior and posterior portions of each sclerotome express different molecular ligands and transcription factors. Those trunk neural crest-derived cells that remain

in the anterior sclerotome form the dorsal root (spinal) ganglia. The path taken by the migrating trunk neural crest cells is controlled by the extracellular matrix (ECM) that surrounds the neural tube. One set of ECM proteins promotes migration of the crest cells. The permissive matrix contains **glycoproteins** (e.g. fibronectin and laminin), various **collagen** molecules and **proteoglycans**. Another set of proteins, the **ephrins**, located in the **posterior section of each sclerotome**, restricts their migration and crest-derived cells always avoid them (Figure 4-17 B, C). (iii) The cells in the third migration pathway continue more ventrally, forming sympathetic ganglia, the adrenal medulla (adjacent to somites 18-24) and nerve clusters surrounding the aorta. Cells from the rostral truncal neural crest also colonize the the esophagus and cardiac stomach.

The vagal (adjacent to somites 1-7) and sacral neural crest (posterior to somite28) will also be the subject of a subsequent lecture (HD 19). The progeny of these cells form the enteric nervous system (Figure 4-16). Vagal crest-derived precursor cells migrate throughout the length of the bowel while

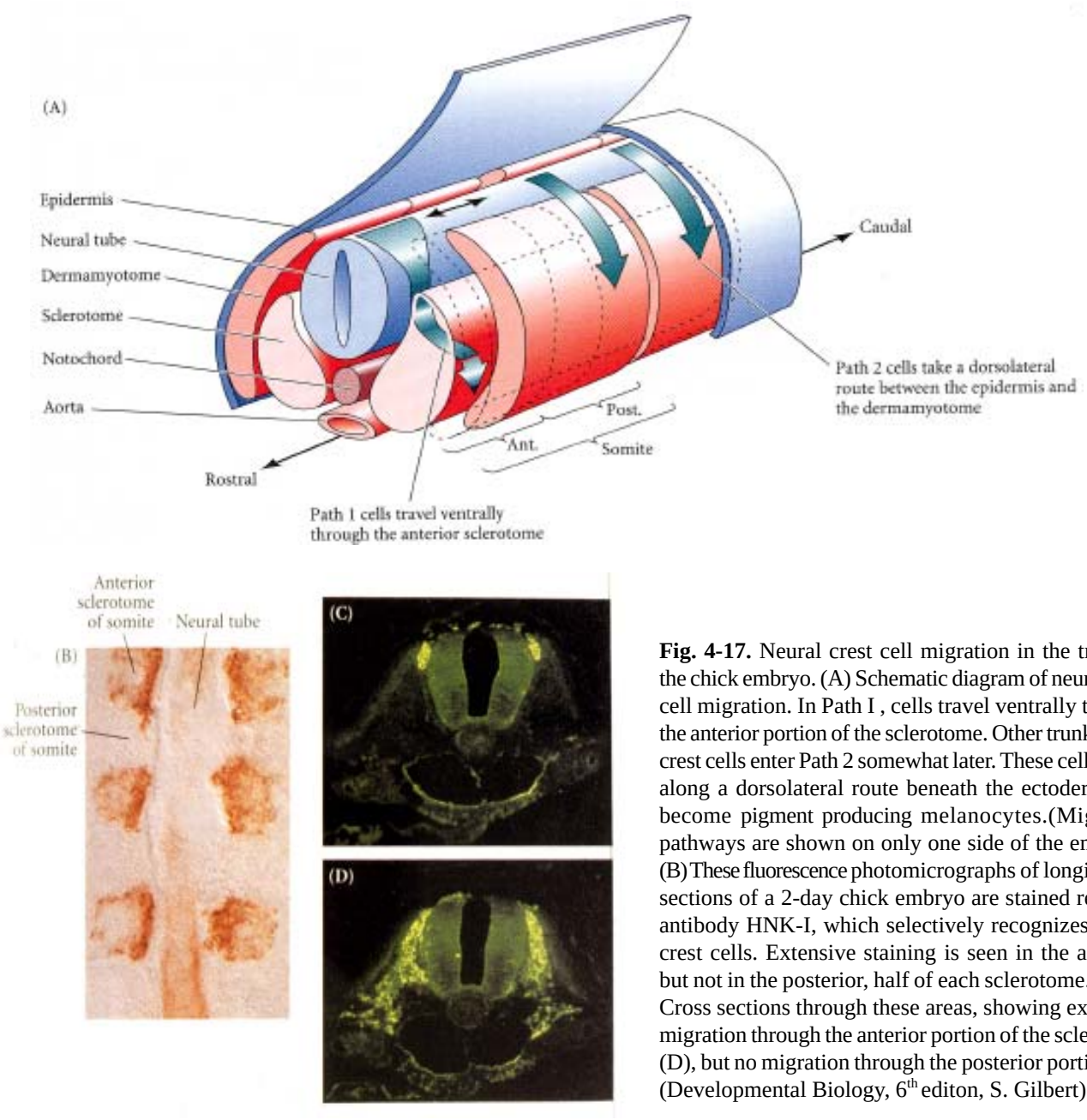

Fig. 4-17. Neural crest cell migration in the trunk of the chick embryo. (A) Schematic diagram of neural crest cell migration. In Path I , cells travel ventrally through the anterior portion of the sclerotome. Other trunk neural crest cells enter Path 2 somewhat later. These cells travel along a dorsolateral route beneath the ectoderm, and become pigment producing melanocytes.(Migration pathways are shown on only one side of the embryo.) (B) These fluorescence photomicrographs of longitudinal sections of a 2-day chick embryo are stained red with antibody HNK-I, which selectively recognizes neural crest cells. Extensive staining is seen in the anterior, but not in the posterior, half of each sclerotome. (C, D) Cross sections through these areas, showing extensive migration through the anterior portion of the sclerotome (D), but no migration through the posterior portion (C). (Developmental Biology, 6th editon, S. Gilbert)

those from the sacral crest are restricted to the post umbilical gut. Failure of neural crest-derived cells to reach the colon from these regions results in the absence of enteric ganglia and thus to the absence of peristaltic movements in the affected portion of colon (Hirschsprung's disease).

The cardiac neural crest is located between the cranial and trunk neural crest. In chick embryos this region extends from the first to the third somites, overlapping the anterior portion of the vagal neural crest (Figure 4-16). During the fifth week of gestation, human cardiac neural crest cells migrate through pharyngeal arches 4 and 6 to form the septum that separates the pulmonary artery from the aorta (Figure 4-18). In chicks, ablation of the neural crest between somites 1-3 results in a single outflow tract emerging from both ventricles. In mice, mutations of the transcription factor Pax3 result in defects in the thymus, thyroid and parathyroid glands as well as a common cardiac outflow tract, demonstrating a role for Pax3 during cranial neural crest migration.

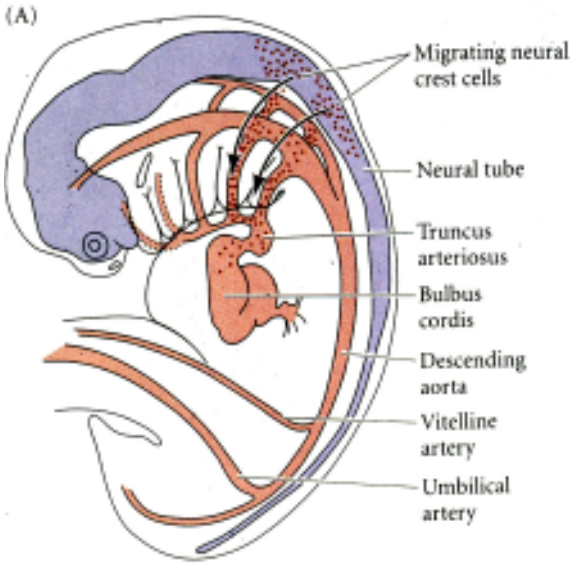

Fig. 4-18. Separation of the truncus arteriosus into the pulmonary artery and aorta. The truncoconal septum (between the aorta and the pulmonary trunk) forms from the cells of the cardiac neural crest. Human cardiac neural crest cells migrate to pharyngeal arches 4 and 6 during the fifth week of gestation and enter the truncus arteriosus to generate the septum (Larsen, 3rd edition, Fig.7-29).

Genetic potential, developmental restriction, differentiation:

Developmental regulation of neural crest cell differentiation requires activation and expression of appropriate transcription factors and receptors. Some populations of neural crest-derived cells are pluripotent and although they are capable of generating a remarkable number of differentiated cell types, their phenotypic repertoire is limited to the expression of those gene products that are appropriate for the target to which they have migrated. Heterotopic transplantation of these cells reveals their greater phenotypic capacity. Other crest-derived cells may constitute a more restricted population of stem cells. There are only a limited number of options in their genetic repertoire. Finally, some pre-migratory crest cells appear to be programmed for a specific developmental fate or if they are not committed to one before leaving the neuraxial crest, they are inhibited from further developmental expression during their migration.