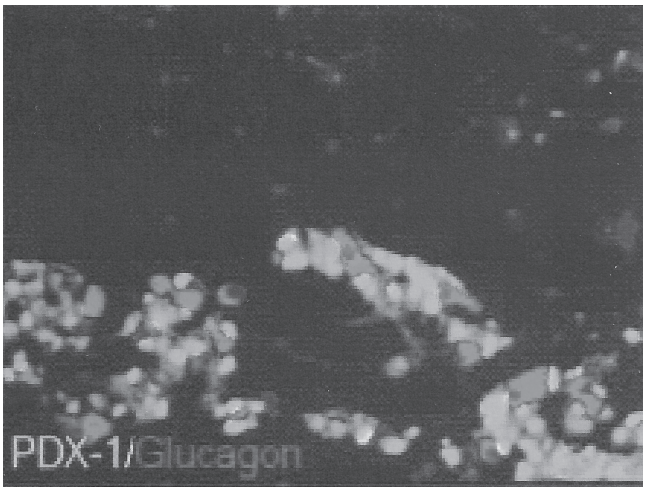
8

EMBRYONIC ORGAN DEVELOPMENT



Early development of the pancreatic islets. By embryonic day 19 (E19), Pdx1-positive (green) and glucagon-positive (red) cell aggregates can be detected in the pancreas. Pdx1 is a marker for the pancreatic β cells, whereas glucagon is expressed in the α cells. The α cells are found primarily around the β cells. (From Jensen J: *Dev Dyn* 229:176–200, 2004, reprinted by permission of Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

DEVELOPMENT OF ECTODERM-DERIVED ORGANS

The *ectoderm* is the outmost layer of the three embryonic germ layers. During the early period of embryogenesis, the ectoderm develops into a structure composed of three functional components: surface ectoderm, neural crest, and neural tube. The *surface ectoderm* is the origin of the epidermis, epidermal appendages (the nails, hair, and sebaceous glands), mucous membrane of the mouth and anus, tooth enamel, lens and cornea, and anterior pituitary. The *neural crest* gives rise to the peripheral nervous system (the peripheral ganglia, Schwann cells, and sympathetic and parasympathetic nervous systems), adrenal medulla, melanocytes, and tooth dentine. The *neural tube* develops into the brain, spinal cord, retina, and inner ear. In this chapter, we will focus on the development of the epidermis and the nervous system.

Epidermal Development [8.1]

The surface ectoderm is originally a structure with a single cell layer, which further develops into a double-layered structure. The external layer forms a temporary tissue that protects the internal layer. The internal layer contains epidermal stem cells and is referred to as the *basal layer.* The basal layer cells can differentiate into all epidermal cell types. As a first step, basal layer cells differentiate into granular cells, which contain keratin granules in the cytoplasm. The granular cells can further differentiate into epidermal cells, also known as *keratinocytes.* These cells can produce a large amount of keratin. Mature keratinocytes move to the external layer of the epidermis and eventually die, forming a keratin-rich layer (Fig. 8.1). This keratin layer is about 10 cells thick and is a tough structure that protects the internal tissues and organs from physical and chemical injury. The dead, keratin-containing keratinocytes on the epidermal surface shed constantly throughout the lifespan. The keratin layer is replenished by newly generated keratinocytes from the granular cells.

The basal cells of the epidermis reside on an extracellular matrix membrane known as the *basal lamina* (Fig. 8.1). Underneath the basal lamina lies the *dermis*, a soft connective tissue. It is important to note that the dermis is not derived from the ectoderm, but from the mesoderm. A major cell type in the dermis is fibroblast. This cell type plays a role in regulating the differentiation and proliferation of the epidermal cells by releasing growth factors, such as fibroblast growth factor, transforming growth factor α, and epidermal growth factor. Keratinocytes can also produce these growth factors. The level and timing of growth factor release may determine the rate of differentiation and proliferation of the basal cells and granular cells.

The *epidermal appendages*, including the hair, nails, and sebaceous glands, are also derived from the basal layer stem cells at designated sites. The formation of epidermal appendages is regulated by epidermal and dermal factors. For hair formation, it is necessary to establish a hair follicle primordium, the early form of the hair follicle. The follicle primordium is derived from the basal layer. At a specific site where dermal fibroblasts are activated by autocrine regulatory factors, the basal layer cells form cell clusters, undergo shape changes, and invade the dermal layer, forming scattered cell nodes. The dermal fibroblasts release growth factors, which stimulate the node cells to divide and differentiate into hair follicle cells and keratin hair shaft. The hair shaft grows out of the dermal layer and forms hair. There are follicle stem cells that can self-renew and differentiate into hair cells and regenerate the hair shaft when hair is damaged, removed, or shed.

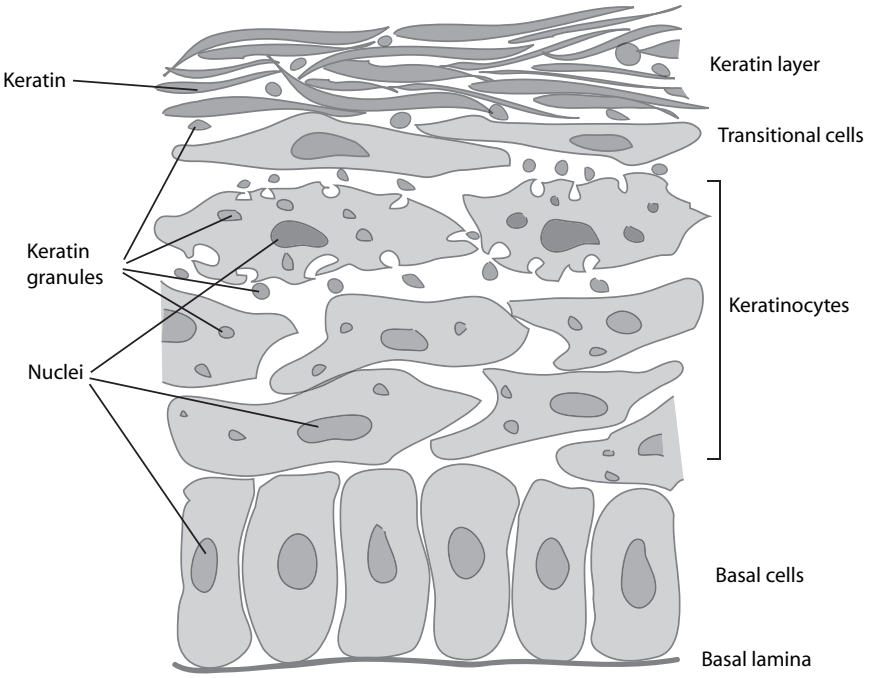


Figure 8.1. Schematic representation of the epidermis. Based on bibliography 8.1.

When the basal layer cells migrate into the dermal tissue for the formation of the hair follicle, a group of cells branches off and forms the *sebaceous glands*, which generate sebum, a lubricant that covers and protects the skin. When the sebaceous gland cells are injured, the follicle stem cells can be stimulated to differentiate into sebaceous cells and replenish the sebaceous glands.

Development of Neural Crest-Derived Systems [8.2]

The Neural Crest. The *neural crest* is an embryonic structure derived from the ectoderm. It contains stem cells that can differentiate into a number of specified cell types and tissues. These include the peripheral ganglionic neurons and glial cells, Schwann cells, sympathetic and parasympathetic neurons, medulla of the adrenal gland, epidermal pigment cells, facial bones and cartilages, corneal endothelial cells, tooth papillae, connective tissue cells and smooth muscle cells in the aortic arch, and connective tissue cells in the salivary and thyroid glands. Since these cells and structures are distributed through the entire body, the neural crest cells must migrate from the ectodermal neural crest to the periphery for a long distance. The control of the migration pattern and destination of neural crest cells as well as the specification and differentiation of these cells are major topics of developmental research.

Neurulation and Formation of Neural Crest Cells. During the stage of blastocyst formation and gastrulation, certain cells in the ectodermal region are prompted to differentiate into *neuroblasts*, cells that develop into nerve cells. This differentiation process is induced

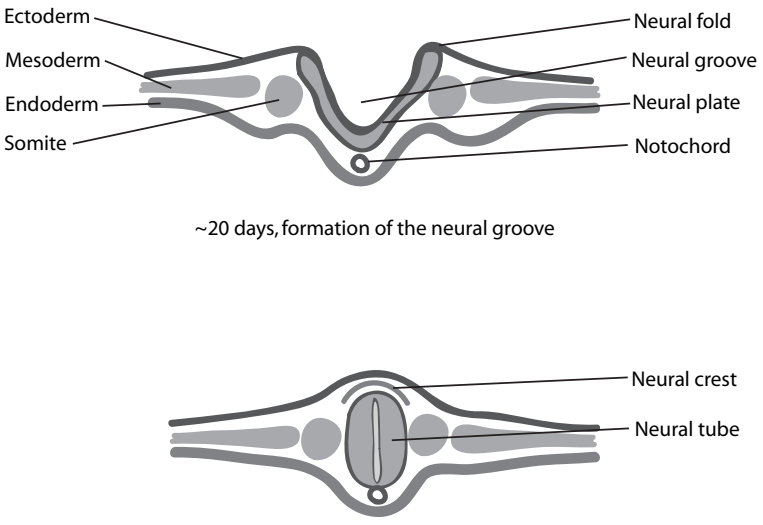


Figure 8.2. Schematic representation of the formation of the neural plate and neural tube, a process known as *neurulation*. Based on bibliography 8.2.

by exposing blastomeres to specific signaling molecules, such as fibroblast growth factor, noggin, and chordin. Soon following gastrulation, the narrow dorsal region of the ectoderm along the anterior–posterior (anteroposterior) axis (superior-inferior axis in the human) is transformed into a neuroblast-containing structure called *neural plate*. The neural plate undergoes a process called *neurulation*, by which the neural plate forms a *neural tube* (Fig. 8.2). There are two forms of neurulation: primary and secondary. *Primary neurulation* is the formation of the neural tube based on the ectoderm neuroblasts. This process takes place in the anterior neural plate, which develops into the brain and anterior spinal cord. In contrast, *secondary neurulation* is the formation of the neural tube from the mesenchymal cells of the mesoderm. The secondary process occurs near the posterior end of the neural plate, which develops into the posterior portion of the spinal cord.

Primary neurulation divides the ectoderm into three layers: the external presumptive epidermis, the middle neural crest cells, and the internal neural tube. At the beginning of the primary neurulation, cells in the neural plate elongate in the direction perpendicular to the neural plate and proliferate symmetrically with respect to the anterior–posterior axis. The neural plate thickens along its edges, bends its edges upward around the anterior–posterior axis, forming the *neural folds*. The groove between the two edges is defined as the *neural groove*, which is the centerline of the left and right central nervous system. The two neural folds bend further, meet each other at the ends, and fuse together to form a tube-shaped structure known as the *neural tube*. The neural tube pinches off from the ectoderm, which becomes an independent structure. At the same time, the fused ectoderm transforms to the epidermal layer at the external surface and the *neural crest* between the epidermal layer and the neural tube.

Secondary neurulation in the posterior portion originates from the mesenchymal cells, which develop into a tube-shaped structure underneath the ectoderm. This tube is

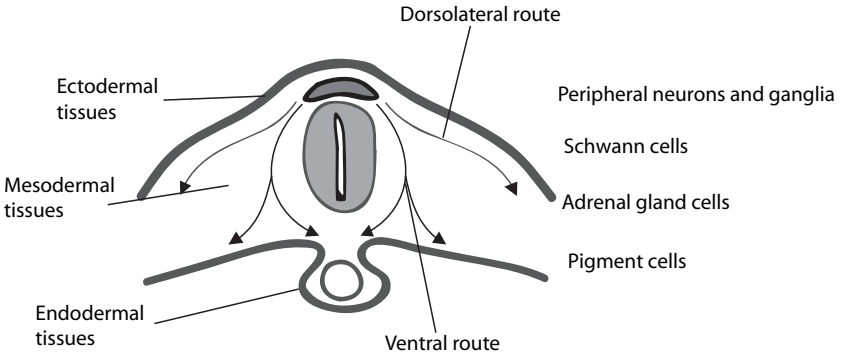


Figure 8.3. Migration routes of the trunk neural crest cells. Based on bibliography 8.2.

connected to the anterior neural tube established by primary neurulation. Secondary neurulation occurs in the region of the lumbar vertebrae. This process gives rise to the posterior spinal cord.

Migration of Neural Crest Cells. During neurulation, the neural crest cells are organized into four groups: cranial neural crest, trunk neural crest, cardiac neural crest, and vagal neural crest. Cells in the *cranial neural crest* migrate to the craniofacial region, forming cranial neurons and glial cells, craniofacial bones, cartilages, and connective tissue. The structures of the head, except for the brain, are developed mostly from the cranial neural crest cells. The *trunk neural crest* cells migrate to the peripheral systems to differentiate into several cell types, including the sensory neurons, medulla of the adrenal gland, sympathetic ganglia, and melanocytes (Fig. 8.3). The *cardiac neural crest* cells migrate to the neck region, the ascending aorta and aortic arch, and the septum to transform into nerve cells, connective tissue cells, the medial and adventitial cells of the ascending aorta. The *vagal neural crest* cells migrate to peripheral systems, primarily the gastrointestinal tract, to form parasympathetic ganglia.

The migration and differentiation of neural crest cells are fundamental processes involved in the formation of almost all body systems. How neural crest cells migrate and differentiate has become a major research topic. While these processes remain poorly understood, previous investigations have provided limited information into the mechanistic aspect. For the migration of the neural crest cells, it is necessary to initiate the following actions: activation of the cell contractile apparatus, dissociation of the cell junctions, and development of extracellular matrix pathways that lead cell migration. Cell movement is initiated by activation of the actin-dependent contractile system. Soon after the neural crest is established, the cells undergo enhanced actin polymerization, resulting in the formation of contractile actin filaments. These filaments are attached to the cell membrane. Their interaction with the myosin molecules induces cell movement. In addition, actin polymerization occurs at the leading edge of migrating cells. Such a process pushes the cell leading edge forward, enhancing cell migration. At the same time, a cell–cell dissociation process is initiated by degrading adhesion molecules such as N-cadherin, which links cells together at the cell–cell junctions. Such a process frees the cells, allowing them to migrate away from the neural crest.

The establishment of appropriate extracellular matrix pathways is another critical factor for directing the migration of the neural crest cells. There are two types of extracellular matrix involved: stimulatory and inhibitory matrix components, which coordinately navigate the migration of neural crest cells. Stimulatory matrix components include collagen, fibronectin, laminin, tenascin, and certain proteoglycan molecules. When encountering cells, these matrix components interact with corresponding integrins on the cell membrane, activating intracellular signaling pathways and promoting cell migration. In a complex biological system composed of a variety of cellular and extracellular components, directed cell migration can only be achieved in the presence of both stimulatory and inhibitory matrix, which confines cells to the stimulatory matrix pathway. Ephrins are a family of inhibitory matrix proteins. These proteins, when interacting with their receptors on the neural crest cells, prevent cell migration. In all tissue and organ systems, stimulatory and inhibitory extracellular matrix components are coordinately organized, ensuring directed migration of neural crest cells.

Differentiation of Neural Crest Cells. *Neural crest cells* are pluripotent cells that can differentiate into a variety of specified cell types essentially in all organ systems. There are several factors that are known to regulate the differentiation of the neural crest cells, including (1) the local environment within the neural crest and (2) the environment of peripheral tissue to which the neural crest cells migrate. Within the neural crest, there exist heterogeneous populations of pluripotent cells. The specification of these populations may be dependent on the local presence of different soluble factors (e.g., bone morphogenetic protein and Wnts) in the neural crest. Different combinations of these factors may determine the fate of the neural crest cells. For instance, when selected trunk neural crest cells (developing into sympathetic neurons) are removed and transplanted to the location of the vagal crest cells (differentiating into parasympathetic neurons) in the chick, the trunk crest cells can generate neurons that produce parasympathetic neurotransmitters. Thus, the initial specification of the neural crest cells is predetermined not by factors within the cells, but by extracellular factors. However, it remains poorly understood how early blastomeres elect to secrete different factors at different locations. (See Table 8.1.)

When neural crest cells migrate out of the neural crest, the fate of the cells is not finally specified. Neural crest cells committed to each pathway, such as the cranial, trunk, vagal, or cardiac pathway, have the potential to develop into different cell types. The local environment of the destination tissue determines the final fate of the neural crest cells. Soluble factors secreted by local cells may play a critical role in cell specification. These factors may include bone morphogenetic factors, glial growth factor, fibroblast growth factor, epidermal growth factors, and platelet-derived growth factor. The ratio of different factors as well as the timing of factor secretion may all contribute to the regulation of cell specification and differentiation.

Development of Neural Tube-Derived Systems [8.3]

Fate of the Neural Tube. The *neural tube* is a straight cylindrical structure located along the anterior–posterior axis of vertebrates (superior–inferior axis in the human) and is formed during neurulation as discussed in the previous section. The neural tube gives rise to the brain and spinal cord as well as their cavities. The anterior portion of the neural tube forms three major parts of the brain: the prosencephalon (forebrain), mesencephalon

TABLE 8.1. Characteristics of Selected Molecules that Regulate the Development of Neural Cells*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Wnt 2	Wingless-type MMTV integration site family member 2, Wnt 2 protein	360	40	Embryo, fetus, thalamus	Regulating embryonic development and promoting oncogenesis
Bone morphogenetic protein 2	BMP2	396	45	Embryo, fetus, bone, cartilage	A member of the transforming growth factor β superfamily, regulating the formation of bone and cartilage
Bone morphogenetic protein 4	BMP4	408	47	Embryo, fetus, cartilage, bone, heart, lung, intestine, ovary	Regulating embryonic development and endochondral bone formation after birth
Bone morphogenetic protein 6	BMP6, VGR1	513	57	Embryo, fetus, cartilage, bone	Regulating embryonic development and inducing bone formation after birth

*Based on bibliography 8.2.

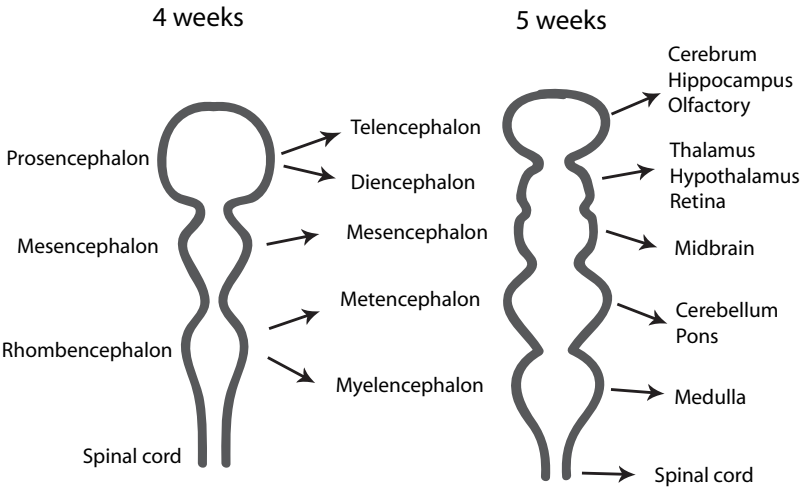


Figure 8.4. Schematic representation of brain development. Based on bibliography 8.3.

(midbrain), and rhombencephalon (hindbrain) (Fig. 8.4). The *prosencephalon* develops into the cerebrum, thalamus, hypothalamus, and retina. The *mesencephalon* gives rise to the cerebral aqueduct. The *rhombencephalon* forms the medulla oblongata and cerebellum. The posterior portion of the neural tube develops into the spinal cord.

Formation of Neurons and Glial Cells. There are two types of cell in the central nervous system: neurons and neuroglial cells. *Neurons* are the cells that can learn, memorize, sense signals from peripheral cells, and control the activity of other cell types. Each neuron is composed of a long axon, which physically connects the central and the peripheral nervous systems and is responsible for signal transduction and information transmission between the central and peripheral systems. In addition, each neuron consists of a large number of short processes known as *dendrites*, which contact other neurons within the central nervous system and are responsible for neuron–neuron communication.

Neuroglial cells serve to support and protect the neurons and to assist in neuronal development. There are three types of neuroglial cell: oligodendrocyte, astrocyte, and microglial cell. The *oligodendrocyte* can form large membrane processes called myelin sheaths, which wrap around the neuronal axons. The myelin sheaths have several functions: axon protection, molecular transport into and from the axon, maintenance of the ionic environment of the neuron, and assistance in the transmission of the action potential. The *astrocyte* also contributes to myelin formation and assists in neuronal function. The *microglial cell* behaves as a phagocyte type that degrades and removes debris from apoptotic cells.

The neuron, oligodendrocyte, and astrocyte are derived from the neural stem cells, defined as *germinal neuroepithelial cells*, in the neural tube. The local environment, to which a neural stem cell is migrating, is a critical factor that regulates the differentiation of the neural stem cells. It is believed that a neural tube cell can develop into either a neuron or glial cell depending on the local stimulation. However, the mechanisms remain poorly understood. Although the microglial cell appears as an interstitial cell type in the

central nervous system, it is not derived from the ectodermal neural tube, but from the mesoderm. Mesodermal cells can migrate into the central nervous system and form microglial cells during development.

In the neural tube, neural stem cells are organized into a single-cell layer and are aligned in the direction perpendicular to the surface of the neural tube. These cells often conduct nonsymmetric division along their axial direction. The daughter cells adjacent to the luminal surface of the neural tube remain to be stem cells, whereas the daughter cells adjacent to the external surface differentiate into committed neural progenitor cells. With continuous development, the neural tube is organized into a structure with three zones: ventricular, intermediate, and marginal zones. The *ventricular zone* contains neural stem cells, the *intermediate zone* contains committed neural progenitor cells, and the *marginal zone* contain neurons and neuronal axons, depending on the subsystems of the brain such as spinal cord, cerebellum, or cerebrum. From the ventricular zone, the committed neural progenitor cells migrate outward into the intermediate zone. In the intermediate zone, different parts of the central nervous system are established. The patterns of cell migration, division, and organization vary considerably between the spinal cord, cerebellum, and cerebrum. The formation of these structures is briefly discussed here.

For the *formation of the spinal cord*, cells in the intermediate zone form axons, which extend from the intermediate zone toward the marginal zone, where the density of neurons reduces compared with the intermediate zone. With further development, established neuroglial cells migrate into the marginal zone and form myelin sheaths that enclose the axons. The neuron-containing mantle zone eventually develops into the butterfly-shaped *gray matter* of the spinal cord, whereas the axon-containing marginal zone forms the peripheral *white matter.* The terms of gray matter and white matter are defined based on the color of the structures under an optical microscope. In the spinal cord, neurons localized to different regions possess distinct functions. Neurons in the dorsal half of the spinal cord are responsible for receiving and processing sensory signals from peripheral neuronal sensors, whereas the ventral neurons are for the motor control function.

For the *formation of the cerebellum*, the pattern of neural cell migration and organization differs from that for the spinal cord. The neuronal stem cells for the development of the cerebellum are evolved to form several types of cerebellar cells, including neurons that form cerebellar nuclei, granule neurons, and Purkinje neurons. For the formation of neuronal nuclei, the neuronal progenitor cells from the neural tube migrate out of the neural tube, establish the intermediate zone, and continue to migrate forward to enter the marginal zone, where they form *neuronal nuclei*, serving as relay units between different systems of the cerebellum and between the cerebellum and other systems of the brain. Another group of neuronal progenitor cells migrates through the intermediate zone, enters the marginal zone, and forms a new layer called the *external granule layer* adjacent to the external surface. Cells in the external granule layer make a turn and migrate inward. At the interface between the marginal and intermediate zones, these cells form the granule neurons. In addition, the neural tube stem cells differentiate into progenitor cells for the Purkinje neurons. These cells migrate to the marginal zone, forming a Purkinje cell layer. The Purkinje neurons are responsible for communications between different systems of the cerebellum.

The *cerebrum* is originated from the anterior portion of the neural tube and organized into a layered structure. During cerebral formation, cerebral progenitor cells are generated from the stem cells located in the ventricular zone, pass through the intermediate zone,

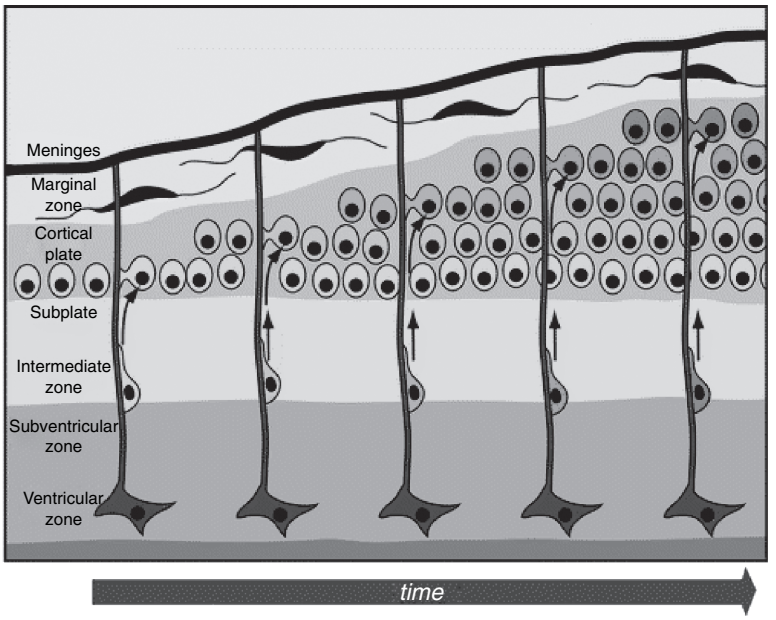


Figure 8.5. Migration and formation of cortical neurons. During development, neurons are generated in the ventricular zone, migrate along radially aligned glial cells through the subventricular zone, intermediate zone, and subplate, and are deployed to the cortical plate. (Reprinted by permission from Bielas S et al: *Annu Rev Cell Dev Biol* 20:593–618, copyright 2004 by *Annual Reviews*, www.annualreviews.org.)

and enter the marginal zone, where they form the cortical plate or the neocortex (Fig. 8.5). The neural progenitor cells in the cortical plate proliferate and differentiate into several layers of neurons in the vertical direction (perpendicular to the surface of the neural tube). Cells in different layers are responsible for the control of different peripheral systems. The cerebral neurons are also organized into a large umber of regions in the horizontal direction. Each region controls a corresponding peripheral system. In the cerebrum, neurons are concentrated in the cortical zone. This zone is referred to as the gray matter based on the color of the neuron-rich brain tissue. The intermediate zone is primarily composed of axons, which appear whitish under an optical microscope, and is referred to as the white matter.

DEVELOPMENT OF MESODERM-DERIVED ORGANS

The *mesoderm* is the middle layer of the three embryonic germ layers established during gastrulation and is the origin of a number of systems, including the cardiovascular system, lymphatic system, skeletal muscle system, bone, cartilage, connective tissue, kidney, and gonads. The mesoderm is composed of four major structures: the notochord (chordamesoderm), paraxial mesoderm, intermediate mesoderm, and lateral plate mesoderm. The organization, development, and fate of these structures are outlined here.

The Notochord [8.4]

The *notochord* is an embryonic structure established during gastrulation and located underneath the neural tube. The notochord is formed from an early embryonic structure known as the *primitive streak*, which is initiated at the posterior end of the embryo in the blastocyst stage with two blastoderm layers (epiblast and hypoblast) and plays a critical role in the formation of the mesoderm and the endoderm. The primitive streak progresses toward the anterior end along the anterior-posterior axis. At the same time, the centerline of the primitive streak depresses, forming the *primitive groove*. When the primitive streak reaches the anterior end, there forms a node structure called *Hensen's node* at the anterior end (superior or cranial end in humans) of the primitive streak. The mesoderm and endoderm are formed by the primitive streak cells, which pass through the primitive streak layer, move from the dorsal to the ventral direction, and spread on the inner surface of the epiblast, forming the mesoderm and the endoderm. These cells form two layers ventral to the epiblast: the middle layer and the internal layer. The former develops into the mesoderm and the later endoderm. A group of primitive streak cells migrates through the Hensen's node and forms the notochord along the anterior–posterior axis. During such a process, the epithelial type of primitive streak cells is transformed into mesenchymal cells. The notochord is a transient structure, which mediates the formation of the neural tube and defines the anterior–posterior axis of the body. In mammals, the notochord cells eventually form part of the endoderm and contribute to the formation of the primitive gut.

The Paraxial Mesoderm

Formation of the Somites [8.5]. The *paraxial mesoderm*, also known as the somatic dorsal mesoderm, is formed by cells from the primitive streak as described above and organized into two parallel columns that are aligned along the neural tube one at each side. The paraxial mesoderm forms segmented blocks called *somites*, also known as *mesodermal segments.* These somites develop into the vertebrae, ribs, skeletal muscles, and dermis. These structures serve as pathways for the extension of the spinal nerve axons and the migration of neural crest cells, which develop into the sympathetic and parasympathetic neurons.

The formation of the somites begins at the anterior portion of the primitive streak. As a first step, paraxial mesodermal cells are organized into two unsegmented presomitic cell clusters called *somitomeres*, which are aligned along the neural tube one at each side. The somitomere at the anterior end is first separated into individual somites. The tip portion of the somitomere adjacent to an established somite is subsequently separated from the main body of the somitomere. The segmentation process continues from the anterior to the posterior directions (superior to inferior direction in humans) until the entire somitomere is segmented. The segmentation of the somitomere is regulated by several molecules, including hairy 1, ephrin, and ephrin tyrosine kinase receptor. Hairy1 encodes a transcription factor, which can activate the expression of the ephrin and ephrin tyrosine kinase receptor genes. In the somitomere, hairy 1 is expressed in the posterior region of a premature somite, leading to local activation of ephrin and ephrin tyrosine kinase receptor. Ephrin can interact with the ephrin tyrosine kinase receptor, triggering the activation of inhibitory signaling pathways. Such an activity results in local suppression of cell interaction with substrate and enhancement of somite separation

from the somitomere. This pattern of molecular activities repeats along the somitomere from the anterior to the posterior direction until the completion of the whole set of somites. The primitive streak is gradually diminished during the segmentation of the somitomere.

Each pair of somites alongside the neural tube is the origin of the muscular and connective tissue specified for a designated location. Each somite, once established and matured, is committed to the specification of a tissue and its developmental course cannot be altered. This phenomenon has been demonstrated by transplantation of a somite from one location to another. Tissues developed from the transplanted somite will appear in the peripheral location of the tissues that are supposed to form from the original somite. For example, when a somite from the thoracic region is transplanted to the cervical region of a chick, ribs will form in the cervical region on the transplantation side. Overall, somites develop into several types of tissues and structures, including the vertebra, rib, limb, skeletal muscle, connective tissue, and back dermis.

At each level, the somite is organized into distinct regions in the transverse direction, including the ventral region, two lateral regions, and dorsal region. The *ventral region* develops into a group of mesenchymal stem cells collectively called the *sclerotome*. Cells in this structure give rise to chondrocytes and osteoblasts of the vertebrae, ribs, and other bones. The two *lateral regions*, one adjacent to and the other farthest from the neural tube, develop into *myotomes*, which form myoblasts, precursors of skeletal muscle cells. The lateral myotome adjacent to the neural tube develops into the back skeletal muscles, whereas the lateral myotome farthest from the neural tube form skeletal muscles in the limbs and other body parts. The *dorsal region* of a somite develops into the *dermatome*, which is the origin of the dermal tissue in the back area. The formation of these distinct somite regions is mediated by the activation of paracrine molecules, including sonic hedgehog, Wnt1, Wnt3a, neurotrophin 3, and bone morphogenetic protein 4 and 5. These factors are expressed and released from the structures adjacent to the somites, such as the neural tube and the lateral plate mesoderm. The location- and time-dependent activation of these factors may determine the specification and commitment of the cells in the sclerotome, myotomes, and dermatome.

Formation of the Skeletal Muscle System [8.6]. As discussed above, the cell lineages in the lateral myotomes develop into skeletal muscles. The myotome contains muscular progenitor cells called *myoblasts*. These cells are prompted to enter a process known as *myogenesis*, the generation of muscular cells. Myogenesis is initiated and enhanced by transcriptional factors MyoD and Myf5, which are myogenic regulatory factors. These factors activate the expression of necessary genes that encode proteins for initiating myogenesis.

During myogenesis, one of the genes that are upregulated is the fibroblast growth factor (FGF) gene. In the presence of increased FGF, myoblasts expand extensively via proliferation. These cells also produce and release extracellular matrix components such as collagen and fibronectin. At a certain time, the FGF level is suppressed, leading to a reduction in the rate of myoblast proliferation. The myoblasts attach to the extracellular matrix through the interaction of the cell membrane integrins, such as $\alpha 5\beta 1$, with selected extracellular matrix components, such as fibronectin. All myoblasts within a muscular bundle are aligned in the same direction by the mediation of cell membrane proteins such as cadherins and cell adhesion molecules (CAMs). The interaction between different myoblasts and between myoblasts and extracellular matrix induce an increase in the level of

calcium, which initiates and regulates myoblast fusion, the formation of multinucleated muscular cells from individual myoblasts. This process is also mediated by a metalloproteinase, known as meltrin α, which degrades extracellular matrix components and enhances myoblast fusion.

Formation of the Skeleton [8.7]. The skeleton is composed of bones and cartilages. These structures are developed from two mesodermal systems, including the somites and lateral plate mesoderm, and an ectodermal system, the cranial neural crest. The somites give rise to the trunk skeleton (vertebrae and ribs), the lateral plate mesoderm gives rise to the limb skeleton, and the cranial neural crest gives rise to the craniofacial skeleton. For the ectodermal source of the skeleton, the neural crest cells are transformed from an ectodermal cell type to a mesenchymal type, which forms skeletal tissues.

There are two mechanisms for the formation of the bone: *direct osteogenesis* from soft mesenchymal tissue and *ossification of cartilage*. The craniofacial bones are formed through direct osteogenesis. In such a process, the cranial neural crest cells migrate from the neural crest to the skull, transform from ectodermal cells to mesenchymal cells, undergo extensive proliferation, and form a mesenchymal tissue. A fraction of the neural crest-derived mesenchymal cells differentiates into *osteoblasts* or bone progenitor cells. The osteoblasts can produce and release collagen and specific proteoglycans, which serve as a matrix for the formation of the bone. This matrix can bind and retain calcium, which results in the calcification or *ossification* of the matrix. All osteoblasts within the calcified matrix are considered mature bone cells and are defined as *osteocytes*. Cells adjacent to the periosteum or the surface membrane of the calcified matrix structure retain the features of osteoblasts and can differentiate into osteocytes and produce bone matrix. Several bone morphogenetic proteins, including BMP2, BMP4, and BMP7, play a critical role in osteogenesis by stimulating the differentiation of osteoblasts and the production of bone matrix components.

Most bones, including the vertebrae, ribs, and limb bones, originate from the somites and lateral plate mesoderm, are developed via cartilage formation and ossification of cartilage. There are several steps for bone morphogenesis: (1) formation of chondrocytes, (2) formation of cartilages, (3) initiation of osteogenesis, and (4) mineralization of bone matrix and formation of bone. *Chondrocytes* are formed from mesenchymal progenitor cells derived from the somites and lateral plate mesoderm under the stimulation of local osteogenic factors. The newly formed chondrocytes expand extensively via proliferation and generate extracellular matrix components that are necessary for the formation of cartilage. Extracellular matrix and chondrocytes are integrated and organized to form *cartilage*. Within the cartilage, established chondrocytes are transformed into hypertrophic chondrocytes characterized with an increase in cell volume. These chondrocytes can produce collagen type X and fibronectin. These matrix components absorb calcium and phosphate into the matrix, initiating *matrix mineralization* or *osteogenesis*. The hypertrophic chondrocytes also produce and release vascular endothelial growth factor (VEGF), which stimulates angiogenesis or blood vessel formation within the cartilage. The vascular system supplies oxygen and nutrients to the newly generated bone. The hypertrophic chondrocytes undergo apoptosis shortly after these initiating processes for bone formation. As a last step, mesenchymal progenitor cells are transformed into osteoblasts, which generate and release bone-forming extracellular matrix components and stimulate matrix mineralization. A bone forms when a sufficient amount of calcium and phosphate is deposited to the bone matrix.

The Intermediate Mesoderm: Formation of the Kidney [8.8]

The intermediate mesoderm gives rise to the *urogenital system*, including the kidney and associated duct systems as well as gonads. For kidney development, there are two critical stages: the formation of *transient kidneys* and the formation of *permanent kidneys*. For the formation of the transient kidneys, the intermediate mesoderm initiates the formation of two *pronephric ducts* at the anterior portion of the mesoderm during the early embryonic stage (about 20 days in humans). This process is symmetric with respect to the neural tube from both parts of the intermediate mesoderm. The formation of the pronephric ducts progress continuously toward the posterior end. Established pronephric ducts interact with adjacent mesodermal tissue to form *pronephroi*, which are side branches attached to the anterior portion of the pronephric ducts. When the formation of the pronephric ducts progresses to the middle portion of the mesoderm, the pronephric ducts stimulate the development of *mesonephroi*, which constitute the major *excretory system* of the embryo. The pronephric ducts further progress to the posterior end, where the two pronephric ducts meet to form the *cloaca*, a presumptive structure for the formation of the bladder, the rectum, and the genital system. During the development of the mesonephroi, the anterior end of the pronephric system undergoes progressive degeneration. This degenerative process continues when the posterior end of the pronephric ducts form new mesonephroi. Shortly after the cessation of mesonephros generation, the mesonephroi are degenerated via cell apoptosis. Thus, the mesonephroi serve only as a temporary excretory system.

While the anterior and middle portions of the pronephric system are degenerated, the posterior portion of the system remains. A side branch, known as the *ureteric bud*, forms from each of the two pronephric ducts in the posterior portion. The ureteric bud serves as the presumptive structure for the formation of the two *permanent kidneys*. The ureteric bud interacts with and stimulates surrounding mesenchymal tissue, inducing the formation of the *metanephrogenic mesenchyme* (Fig. 8.6). At the same time, the metanephrogenic mesenchyme stimulates the ureteric bud to extend and branch into the mesenchyme. The ureteric bud-derived branching system serves as a kidney rudiment, which eventually develops into the kidney. At the end of each established ureteric branch, epithelial cells from the metanephrogenic mesenchyme form a cell cluster and undergo proliferation and differentiation, resulting in the formation of specified kidney cell types, including the glomerular capsule cells, endothelial cells, and tubular cells. These cells are assembled into functional kidney units known as the *nephrons*. The cells in each nephron are integrated into the structure at the tip of each ureteric branch, establishing a connection between the nephron and the ureteric bud branch, which eventually forms the renal collecting duct. Urine forms in the nephrons and is conveyed to the ureters via the collecting ducts.

The Lateral Plate Mesoderm

The lateral plate mesoderm is located farthest from the central neural tube and is composed of two layers: the somatic mesoderm and the splanchnic mesoderm. The *somatic mesoderm* is the layer adjacent to the ectoderm, and the *splanchnic mesoderm* is the layer next to the endoderm. These mesodermal structures develop into the heart, blood vessels, and blood cells. There is a gap between the two mesodermal layers, which is known as the *coelom*. The coelom forms the three body cavities, including the pleural (thoracic), pericardial (cardiac), and peritoneal (abdominal) cavities.

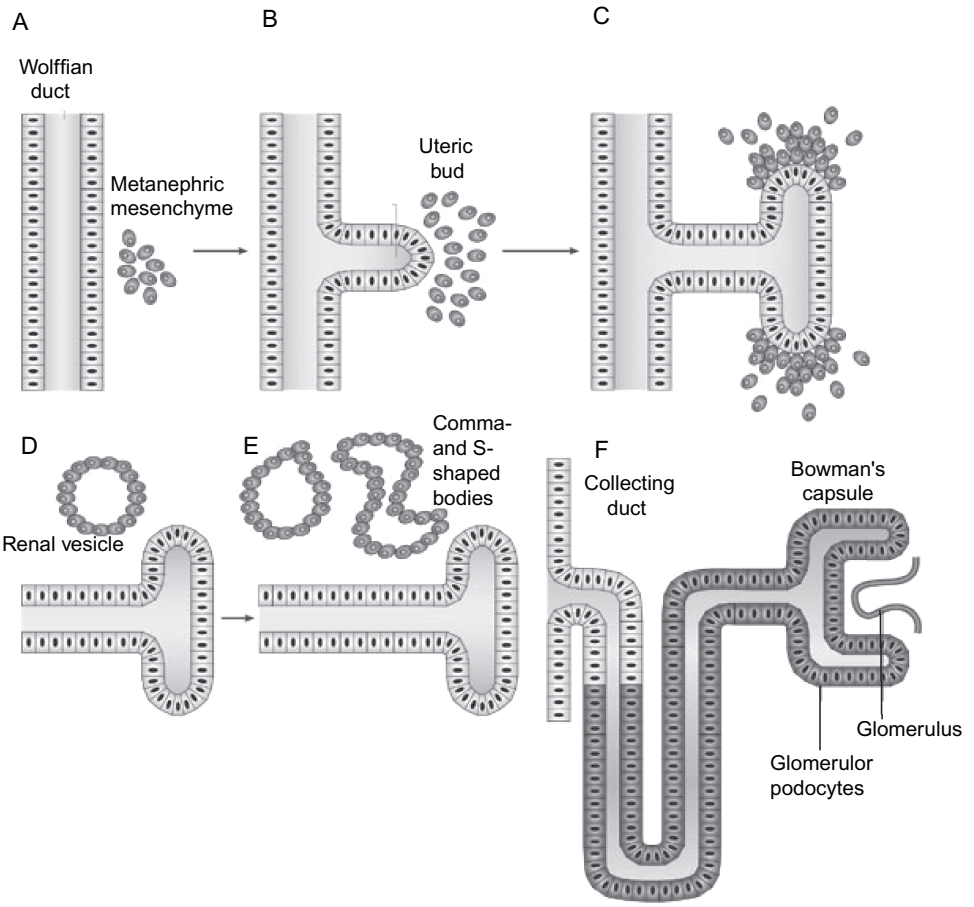


Figure 8.6. Processes of nephron formation. (A) The metanephric mesenchyme interacts with the Wolffian duct of the intermediate mesoderm. (B) At about embryonic day 10.5 (E10.5), the ureteric bud forms from the Wolffian duct and invades the metanephric mesenchyme and induces differentiation. (C) At about E11.5, the mesenchymal cells condense to form aggregates near the ureteric bud. Branches form from the ureteric bud. (D) At about E12.5, the condensed mesenchyme undergoes a mesenchymal-to-epithelial transformation and forms epithelial structures (renal vesicles). (E) At about E13.5, complex epithelial structures, comma- and S-shaped bodies, are formed. (F) From E14.5 to E16.5, the S-shaped bodies fuse into the ureteric bud derivatives, inducing the formation of mature nephron. The ureteric bud develops into collecting ducts. Endothelial cells migrate into the nephron and form the glomerulus, which is surrounded by glomerular podocytes (Bowman's capsule). Selected transcription factors and signaling molecules expressed in the uretic bud and metanephic mesenchyme are shown. WT1 (Wilms' tumor 1), PAX2 (paired box 2), and EYA1 (eyes absent 1) are transcription factors expressed in the metanephric mesenchyme and regulate ureteric bud induction. GDNF (glial-cell-line-derived neurotrophic factor) is also expressed in the metanephric mesenchyme and stimulates ureteric bud induction and branching by interacting with its receptor RET. WT1 and bone morphogenetic protein 7 (BMP7) play a role in regulating the survival of the metanephric mesenchyme. WNT4 is essential for the mesenchymal-to-epithelial transformation of the metanephric mesenchyme and might be induced by WNT6 signals from the ureteric bud. BMP4 is involved in ureteric bud growth, and WNT11 regulates branching of the ureteric bud. GPC3 modulates signals between the metanephric mesenchyme and the ureteric bud, including bone morphogenic proteins. (Reprinted by permission from Macmillan Publishers Ltd.: Rivera MN, Haber DA: *Nature Rev Cancer* 5:699–712, copyright 2005.)

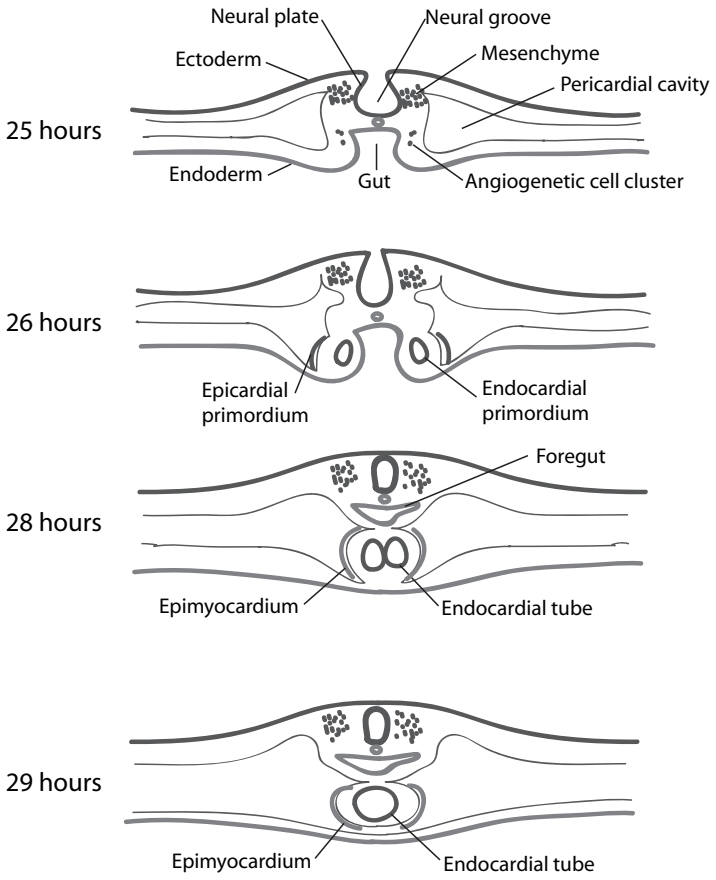


Figure 8.7. Early developmental stages of the chick heart. (After Calson BM: *Pattern Formation of Embryology*, McGraw-Hill, New York, 1981.)

Formation of the Heart [8.9]. The *presumptive cardiac cells* are specified during the early embryonic stage when the primitive streak forms. These cells migrate through the primitive streak and form two *cardiogenic mesodermal clusters*, which are symmetrical with respect to the primitive streak, in the splanchnic mesoderm at the level of the Hensen's node (Fig. 8.7). The two presumptive cardiac cell clusters move toward each other and form two *endocardial primordia*, the initial form of the heart. At the same time, the adjacent mesodermal cells are stimulated to transform into epithelial cells, forming the *pericardium*, which encloses the endocardial primordia, and the endocardium, which lines the internal surface of the heart. Within about 3 weeks in the human, the two endocardial primordia meet and fuse into each other to form a single *myocardial tube*. Cells with cardiomyocyte phenotypes are established during endocardial primordial fusion and initiate the contractile activity, causing pulsations of the endocardial primordia.

Within about 5 weeks, the myocardial tube is developed into a tube with two chambers: the atrium and ventricle (Fig. 8.8). A fraction of cells from the endocardium forms a structure called *endocardial cushion*, which separates the two-chambered tube into the left and right cardiac canals. At about the same time, the atrial septum develops from the

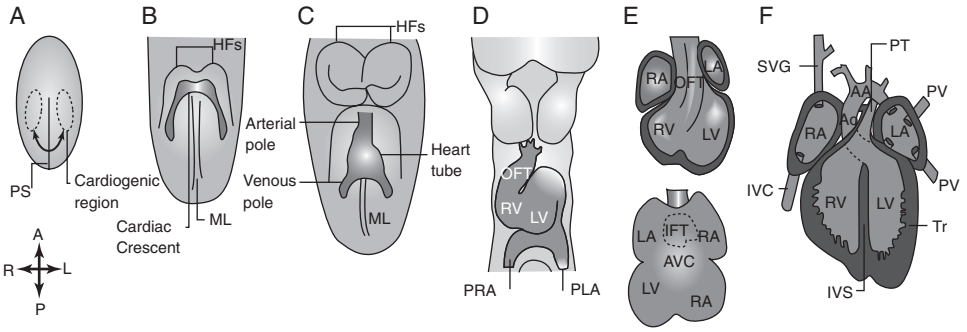


Figure 8.8. Development of the heart. (A) Myocardial progenitor cells originate in the primitive streak (PS) and migrate to the anterior of the embryo at about embryonic day E6.5. (B) The myocardial progenitor cells settle under the head folds (HF) and form the cardiac crescent at about E7.5. (C) The early cardiac tube forms through fusion of the cardiac crescent at the midline (ML) at about E8. (D) The cardiac tube forms a loop at about E8.5. (E) The cardiac tube further develop into chamber structures by about E10.5, but the chambers are still connected. (F) By about E14.5, the cardiac chambers are separated. The left and right ventricles are connected to the pulmonary trunk (PT) and aorta (Ao), respectively. (*Abbreviations*: A, Anterior; P, posterior; R, right; L, left; AA, aortic arch; AVC, atrioventricular canal; IFT, inflow tract; IVC, inferior vena cava; IVS, interventricular septum; OFT, outflow tract; PLA primitive left atrium; PRA, primitive right atrium; PV, pulmonary vein; SVC, superior vena cava; Tr, trabeculae.) Reprinted by permission from Macmillan Publishers Ltd.: Buckinghamm et al: *Nature Rev Genet* 6:826–37, copyright 2005.)

atrial portion of the myocardial tube, while the ventricular septum grows from the ventricular portion. Both septa grow toward the endocardial cushion and separate the myocardial tube into a four-chamber structure—the heart. With further development, the heart is connected to the aorta, pulmonary arterial trunk, and vena cava, which are developed simultaneously with the heart.

The *embryonic circulation* is established when the heart and blood vessels form. The embryonic circulation is different from the *postnatal circulation*. The difference is caused by distinct functional anatomy of the cardiovascular, pulmonary, and intestinal systems between the prenatal and postnatal stages. During the prenatal stage, the embryo or fetus does not have a functional lung and intestine. Oxygen and nutrients are supplied from the mother's blood through the placental circulation. During the stage of the two-chamber heart (about 5 weeks in humans), the umbilical venous blood obtains oxygen and nutrients from the placenta, and the veins conduct oxygenated blood to the heart. The heart pumps blood into the arterial system, which supplies blood to the peripheral systems. The arterial system also conveys blood to the placenta, where metabolic wastes are transported to the mother's circulation.

During the stage of the four-chamber heart when the basic structures of the vascular and other systems are formed, the umbilical veins convey oxygenated blood to the vena cava and subsequently to the right atrium. Unlike the anatomy of the postnatal heart, the right and left atria are connected through the foramen ovale, which is open during the prenatal stage. In addition, the ductus arteriosus between the aorta and pulmonary arterial trunk is also open in the embryo or fetus. Oxygenated blood from the umbilical veins can directly enter the left atrium and left ventricle through the open foramen ovale. The left

ventricle pumps oxygenated blood into the arterial system, which supplies blood to the peripheral systems. Oxygenated blood enters the pulmonary circulation from the right ventricle. A fraction of blood is diverted into the aorta through the ductus arteriosus. Deoxygenated blood with metabolic wastes from the lung returns directly to the left heart, while deoxygenated blood from peripheral organs returns to the right heart. Thus there is a significant degree of mixing between oxygenated and deoxygenated blood in the prenatal circulatory system. Deoxygenated blood with metabolic wastes is conveyed to the placenta for excretion via the umbilical arteries, which bifurcate from the common iliac arteries.

When a baby is born, the umbilical circulation no longer supplies oxygenated blood. A number of anatomical changes take place immediately during the early postnatal period: (1) the lung starts to expand and function, ensuring a transition of gas exchange from the placenta to the pulmonary system; (2) the foramen ovale closes to stop blood diversion from the right to the left atrium; (3) the ductus arteriosus closes to prevent blood diversion from the pulmonary arterial trunk to the aorta; and (4) the umbilical arteries and veins are committed to degeneration. These drastic anatomical changes ensure the establishment of the postnatal circulation.

Formation of the Vascular System [8.10]. Blood vessels are formed from the splanchnic mesoderm, occurring simultaneously with heart generation. As a first step, the mesodermal cells differentiate into *hemangioblasts*, which are progenitor cells for blood vessels as well as blood cells. The hemangioblasts migrate to designated tissues and organs, where they form cell clusters known as *blood islands* (Fig. 8.9). A blood island is composed of two types of cell: *hematopoietic stem cells* (inner cells of the blood island) and *angioblasts* (outer cells). The hematopoietic stem cells develop into blood cell types, including erythrocytes, leukocytes, and platelets, whereas the angioblasts give rise to vascular cells, including endothelial cells, smooth muscle cells, and fibroblasts. There are two basic processes for the formation of blood vessels: vasculogenesis and angiogenesis. *Vasculogenesis* is the formation of primary endothelial cells and capillaries, which occurs during the embryonic stage. *Angiogenesis* is the formation of arteries, veins, and capillaries based on the established capillaries (Fig. 8.10). Angiogenesis occurs not only during the embryonic stage but also during the adulthood in response to injury and pathological disorders, such as cancer and hypertrophy. For vasculogenesis, the angioblasts differentiate into endothelial cells, a cell type that lines the internal surface of a blood vessel. The endothelial cells subsequently form tube-shaped capillaries, which are linked together into a capillary network known as the *primary capillary plexus.* These capillaries are further developed into arteries, veins, and additional capillaries via angiogenesis. During angiogenesis, endothelial cells undergo proliferation and sprout to form new blood vessels. Pericytes are recruited to newly formed capillaries and transformed into smooth muscle cells, eventually leading to the formation arteries and veins.

Several growth factors, including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and angiopoietin, play important roles in the regulation of vasculogenesis and angiogenesis. Fibroblast growth factor is produced by mesodermal cells and released into the interstitial space. This growth factor can stimulate the differentiation of mesodermal cells into hemangioblasts. Vascular endothelial growth factor is also produced by mesodermal cells. This factor directly stimulates angioblasts to differentiate into endothelial cells and stimulates endothelial cells to form capillaries. The effect of VEGF is dependent on the type of its receptor on

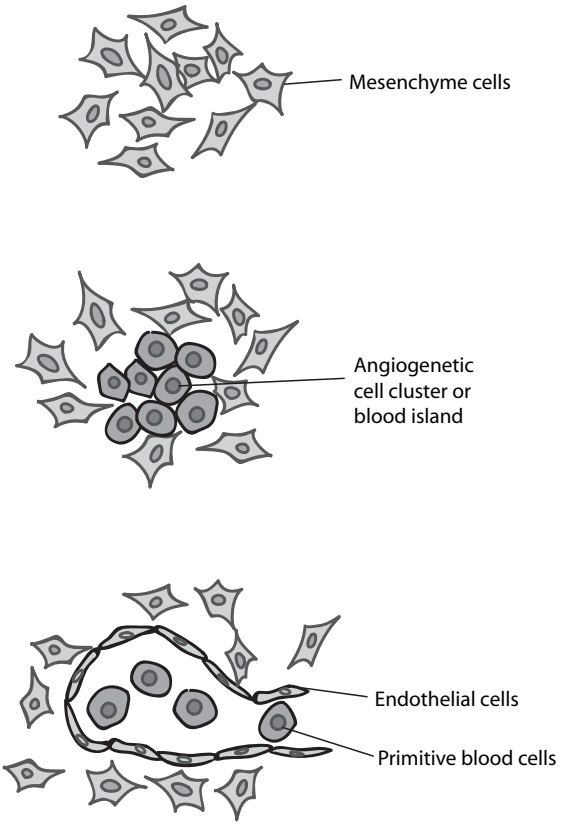


Figure 8.9. Schematic representation of embryonic blood formation and vasculogenesis. (After Langman J: *Medical Embryology*, 4th ed., Williams & Wilkins, Baltimore, 1981.)

the cell surface. There are two types of VEGF receptor: flk1 (VEGF receptor 2) and flt1 (VEGF receptor 1). The activation of flk1 induces the differentiation of angioblasts into endothelial cells. The activation of flt1 results in the formation of endothelial tubes. Platelet-derived growth factor stimulates the recruitment of pericytes to newly formed blood vessels and the transformation of these cells to smooth muscle cells. Another factor, angiopoietin, interacts with its receptor Tie2 and regulates the recruitment of smooth muscle cells and the formation of blood vessels. Thus, these growth factors are essential for both vasculogenesis and angiogenesis.

Formation of Blood Cells [8.11]. *Blood cells* are a family of circulating cells, including erythrocytes, leukocytes (monocytes, neutrophils, basophils, eosinophils, and T and B lymphocytes), and platelets. Blood cells are developed from the hematopoietic stem cells derived from the mesodermal hemangioblasts. The process of blood cell formation is defined as *hematopoiesis*, which is a continuous process through the lifespan because of constant death of blood cells. There are two types of hematopoiesis: embryonic and adult hematopoiesis, which occur during and after the embryonic stage, respectively. During the *embryonic* stage, *hematopoiesis* initially occurs in the blood islands derived from the lateral plate mesoderm (Fig. 8.9). The blood islands, however, are not permanent sites for

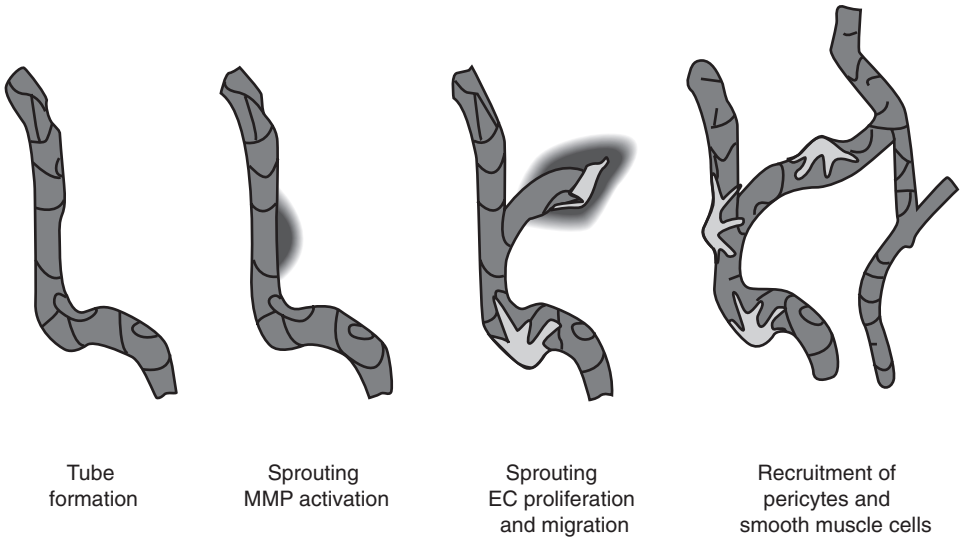


Figure 8.10. Schematic representation of the blood vessel formation, which involves endothelial cell tube formation, endothelial cell proliferation and migration, sprouting angiogenesis, and pericyte recruitment. (Reprinted from Hallmann R et al: *Physiol Rev* 85:979–1000, 2005 by permission of the American Physiological Society.)

hematopoiesis. The site of hematopoiesis changes for several times during embryogenesis. The first change occurs when the blood islands are transformed into blood vessels. The site of hematopoiesis is shifted from the blood islands to structures near the aorta known as the *aorta–gonad–mesonephroi*. With the degeneration and disappearance of the mesonephroi, the site of hematopoiesis is moved to the liver. During the late embryonic stage, hematopoiesis takes place in the bone marrow. During the adulthood, the bone marrow is a permanent site for hematopoiesis. Blood cells die constantly during the lifespan and dead cells are replaced with newly generated cells from the bone marrow.

In the bone marrow, there exist pluripotent *hematopoietic stem cells*, which can self-renew, proliferate, and differentiate into all mature blood cell types during the embryonic stage as well as the adulthood. The percentage of hematopoietic stem cells in the bone marrow is about 0.01%. Such a small fraction of cells can replenish the entire family of blood cells. Hematopoietic stem cells can differentiate into lineage progenitor cells, including B- and T-lymphocyte progenitor cells and myeloid progenitor cells. The *B-lymphocyte progenitor cells* can differentiate into B-lymphocytes and plasma cells through several intermediate cell lineages, including the pro-B cell and pre-B cell lineages. The *T-lymphocyte progenitor cells* can differentiate into T-lymphocytes through cell lineages, including the pro-T cell, pre-T cell, and immature thymocyte lineages. The *myeloid progenitor cells* can differentiate into at least five types of lineages: erythroid precursor (CFU-E), platelet precursor (CFU-MK), monocyte precursor (CFU-M), neutrophils precursor (CFU-G), basophil precursor (CFU-BUO), and eosinophil precursor cells (CFU-Eo). The *erythroid precursor cells* can differentiate subsequently into proerythroblasts, erythroblasts, reticulocytes (with nuclei removed), and erythrocytes. The *platelet precursor cells* can develop into immature megakaryocytes and megakaryocytes, which split into platelets. The *monocyte precursor cells* can give rise to monocytes, which transform

into macrophages when migrating into the blood vessel wall and interstitial space. The *neutrophil*, *basophil*, and *eosinophil precursor cells* can differentiate into neutrophils, basophils, and eosinophils, respectively. Mature blood cells are generated in the bone marrow and transported to the vascular system.

DEVELOPMENT OF ENDODERM-DERIVED ORGANS

The *endoderm* is the innermost layer of the three embryonic germ layers and gives rise to the lining and gland structures of the digestive and respiratory systems, including the gastrointestinal tract, liver, pancreas for the digestive system and the lung and airways for the respiratory system (Fig. 8.11). In addition, the endoderm plays an important role in regulating the formation of the mesodermal tissues and organs, such as the heart and blood vessels, via interaction with mesodermal cells and release of soluble mediating factors. Here, we focus on the formation of the digestive and respiratory systems.

Formation of the Digestive Tract [8.12]

The digestive system develops from an early endodermal structure known as the *primitive gut*. In the human, the primitive gut forms at about 16 days following the conception and is composed of three parts in the early stage: the foregut, the midgut, and the hindgut. At about 22 days, the *liver bud* forms from the foregut and is the presumptive structure for the formation of the liver. At about 28 days, the anterior end of the foregut opens up to form the *oral opening*, which is the presumptive structure of the mouth. The foregut also gives rise to the pharynx, esophagus, thyroid bud, and lung bud, and stomach at about the

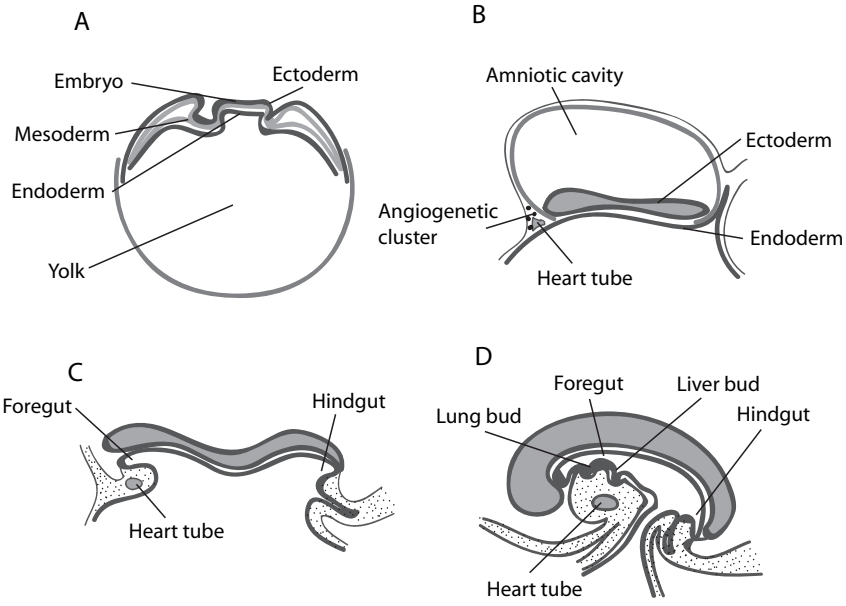


Figure 8.11. Schematic representation of the development of the endodermal organs. Based on bibliography 8.12.

same time. The midgut and hindgut are the presumptive structures for the small and large intestines.

In the anterior region of the primitive gut, there forms the *pharynx primordium*, a structure composed of several clusters of cells known as the *pharyngeal pouches*. These pouches give rise to a number of tissues and organs, including the middle ear, tonsil, thymus, thyroid gland, parathyroid gland, the respiratory tube, and pharynx. Following the establishment of the pharynx, the primitive gut forms subsequently the esophagus, stomach, and small and large intestines (Fig. 8.11). It is important to note that cells in the primitive gut only give rise to the internal epithelial cells and gland cells of the gastrointestinal system. The smooth muscle cells and fibroblasts in the submucosa, muscular, and adventitial layers are derived from the lateral plate mesoderm. The mesodermal cells not only directly contribute to the construction of the digestive tract, but also participate in regulating the specification of the endodermal cells. The interaction of endodermal cells with local mesodermal cells stimulates the specification of the endodermal cells to designated endodermal tissues and organs. Regional expression of different regulatory factors from the mesoderm and endoderm may influence the specification of the endodermal cells.

Formation of the Liver [8.13]

The primitive structure of the liver, known as the *liver bud*, sprouts from the foregut of endodermal primitive gut (Fig. 8.12). The liver bud grows into the surrounding mesodermal tissue, which plays a critical role in the formation of the liver. The mesodermal

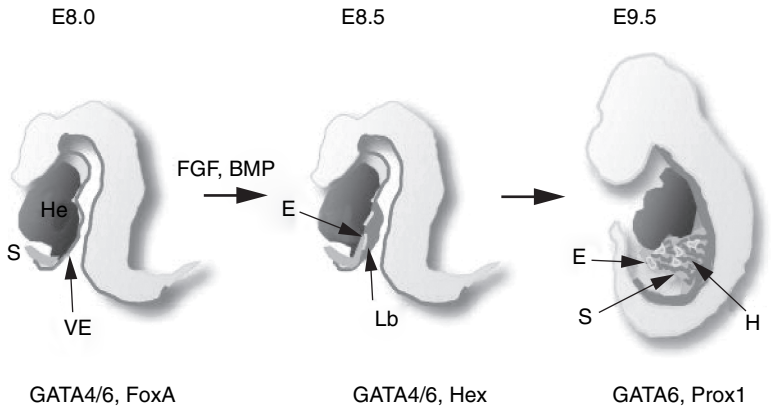


Figure 8.12. Early development of the mouse liver. By about embryonic day 8 (E8.0), a ventral portion of the endoderm near the developing heart (He) is stimulated to initiate the formation of the liver bud (Lb) in response to bone morphogenetic protein (BMPs) and fibroblast growth factor (FGFs). By embryonic day 8.5, the specified hepatic endoderm forms the liver bud, which expresses several liver-specific mRNAs, including that for albumin. Endothelial cells (E) are formed around the liver bud and are necessary for the liver bud development. By embryonic day 9.5, the nascent hepatoblasts (H) delaminate from the ventral endoderm (VE) and invade the septum transversum (S) mesenchyme, which is the source of stellate cells as well as sinusoidal endothelial cells. (Reprinted from Zhao R, Duncan SA: *Hepatology* 41:956–67, 2005 by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

cells produce and release regulatory factors that stimulate the liver bud cells to proliferate and differentiate into various hepatic cells, such as hepatocytes and duct epithelial cells. In particular, the presence of vascular endothelial cells, which form from the mesoderm, is necessary for the formation of the liver. The removal of vascular endothelial cells results in the failure of liver formation. Gallbladder is an affiliated structure of the liver and is formed based on a branch from the hepatic drainage duct.

Formation of the Pancreas [8.14]

The *pancreas* is originated from the endodermal foregut near the liver bud. As a first step, two pancreatic rudiments form at about 30 days in the human: one from the foregut called the *dorsal pancreatic bud* and the other from the hepatic duct called the *ventral pancreatic bud*. These buds subsequently develop into the dorsal and ventral pancreas, respectively, at about 35 days. The two pancreatic structures fuse together to form the pancreas. The formation of the pancreas is mediated by mesodermal cells. Cells from the mesodermal notochord can produce and release soluble regulatory factors, such as fibroblast growth factor 2 and activin, which regulate the differentiation of endodermal cells into pancreatic cells. In addition, vascular endothelial cells are involved in the formation of pancreas. In the absence of vascular endothelial cells, the endodermal cells are unable to differentiate into pancreatic progenitor cells. In particular, endothelial cells can release regulatory factors that mediate the formation of insulin-secreting endocrine cells.

Formation of the Lung [8.15]

The lung is derived from the lung rudiment sprouted from the digestive foregut. The lung rudiment first grows into the trachea, bifurcates into the left and right bronchi, and subsequently establishes the left and right lungs (Fig. 8.13). The mesenchymal cells of the mesoderm interact with the epithelial cells derived from the endoderm and play an important role in the formation of the respiratory system. For instance, when embryonic rat tracheal epithelial cells are cultured in the absence of mesenchyme, the epithelial cells will not develop into airway structures. In contrast, when lung epithelial cells are cultured in the presence of lung mesenchyme, airway-like structures form at the distal end (Fig. 8.14). A regional difference in the characteristics of the mesenchymal cells may determine the specification of different cell types of the respiratory system. The lung is not functional during the embryonic stage, when the embryo or fetus obtains oxygen from the placenta. The lung initiates gas ventilation and exchange immediately after birth.

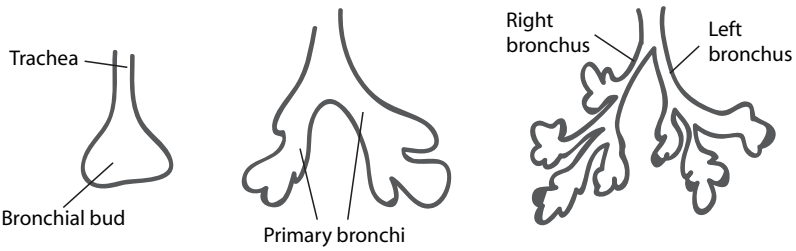


Figure 8.13. Schematic representation of the lung development. Based on bibliography 8.15.

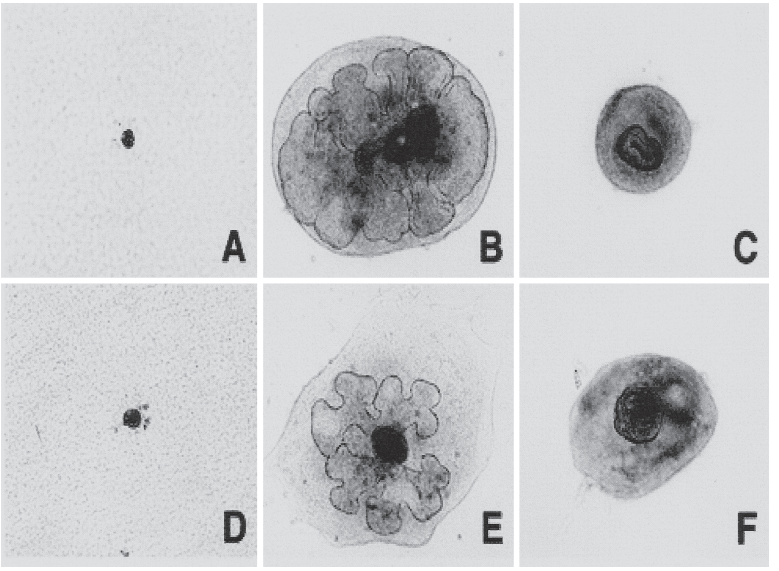


Figure 8.14. The development of the lung is mediated by the interaction of epithelial cells with mesenchymal cells. When embryonic rat lung (A) or tracheal epithelial cells (D) are cultured in Matrigel in the absence of mesenchyme, the epithelial cells will not develop into pulmonary structures. In contrast, when lung epithelial cells are cultured in the presence of lung mesenchyme (B), branching structures form at the distal end. Interestingly, when lung epithelial cells are cultured in the presence of tracheal mesenchyme (C), epithelial cell growth, but not branching, was found. Tracheal epithelial cells also exhibit similar activities (F). When tracheal epithelial cells were cultured in the presence of lung mesenchyme (E), the cells grow and form branches, lading to the development of a lung-like pattern. (Reprinted with permission from Shannon JM, Hyatt BA: *Annu Rev Physiol* 66:625–45, copyright 2004 by *Annual Reviews*, www.annualreviews.org.)

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