CHAPTER 1

Development of the Heart and Great Vessels

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Major advances in molecular genetics, establishment of appropriate animal models, and improvements in analytical techniques have contributed to a greater understanding of cardiac development. Modern cardiac embryology now combines molecular and cellular biologic techniques with traditional embryologic morphologic approaches across multiple model systems. A significant proportion of our understanding continues to be derived from nonhuman experimental models, supplemented by observations imputed from the congenitally malformed human heart [1]. In early studies, avian embryos were the favored experimental model because of the ease with which they could be observed and manipulated. Due to the strength of genetic and molecular investigative tools, the mouse has now become the preferred model for studying cardiac development. Table 1.1 provides a simplified comparison of developmental staging in human, mouse, and chicken embryos [2–8]. Understanding cardiac development not only has implications for classifying and managing congenital heart disease, but also provides a platform for the development of novel management approaches, in both children and adults.

With a goal of simplifying the description of complex developmental structures, in this chapter we have made efforts to harmonize nomenclature using descriptive terms that relate as much as possible to human development. Thus, "anterior-posterior axis" is replaced by "dorsal–ventral axis" or "cranial–caudal." "Anterior" is often replaced by "ventral" or "cranial." "Posterior" is frequently replaced by "dorsal" or "caudal." "Conus" is replaced by "proximal outflow tract," and "truncus" is replaced by distal outflow tract. The "dorsal mesocardial protrusion" is referred to as the "vestibular spine."

Origin of Cardiac Precursor Cells

All cells destined to become part of the heart derive from populations of undifferentiated precursors. These precursor cells are influenced by external signals and guided to their final developmental state. In humans, during the second week following fertilization, the blastocyst has partially embedded into the uterine endometrium. At this stage, the inner cell mass, or embryoblast, differentiates into two distinct layers of cells: a larger columnar epiblast layer and the smaller cuboidal hypoblast layer. The third week of development is characterized by the next critical embryonic process, termed gastrulation. A primitive streak is formed in the epiblast layer, following which some epiblast cells invaginate under and displace the hypoblast. Subsequent widespread cell migration into, and reorganization within, the blastocele cavity results in the formation of three germ layers: the ectoderm, mesoderm, and endoderm. This sets the stage for the determination of the future body plan of the embryo (Figure 1.1) [3,9].

Migrating epiblast cells, which have now formed the mesoderm of the embryo, gradually travel cephalad. During this migration, they join the lateral plate mesoderm at the level of the primitive node. The lateral plate mesoderm then divides into two layers: a splanchnic layer directly above the endoderm, and a somatic layer directly below the ectoderm. The anterior endoderm provides signals to splanchnic mesodermal cells to enter the precardiac lineage. Fibroblast growth factors (FGFs)-1, -2, and -4, and bone morphogenetic protein 2 (BMP-2), are proteins that appear to be critical to this process [10]. To date, however, no single gene has been identified whose ablation leads to a specific failure of all myocardial

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Table 1.1 Simplified comparison of developmental stages between human, mouse, and chicken embryos.

Human			Mouse	Chicken	
Carnegie Stage	Streeter horizon	Days' gestation	Embryonic days (Theiler's stage)	Hamburger/Hamilton stage (days of incubation)	Human cardiac developmental milestone
9	IX	20	8–8.5 (12)	7–8 (1.1)	Circulation begins
10	X	22	8.5–9 (13)	10 (1.5)	
11	XI	24	9–9.5 (14)	11 (1.8)	
12	XII	26	9.5–10.25 (15)	14 (2.2)	
13	XIII	28	10.25–10.5 (16)	17 (2.6)	Looping
14	XIV–XV	32	10.5–10.75 (17)	19 (2.9)	Atrial septation begins
15	XVI	33	11 (18)	20–21 (3.3)	OFT septation begins
16	XVIII	37	11.5 (19)	24 (4)	Ventricular and AV septation begins
17	XX	41	12 (20)	26 (4.8)	
18	XXI–XXII	44	12.5 (21)	28 (5.6)	Valvar maturation
19	XXIII	47	13 (21)	29–30 (6.4)	
20		50	14 (22)	31–32 (7.2)	
21		52	14 (22)	34 (8)	
22		54	14 (22)	35 (8.7)	
23		56	14 (22)	36 (9.6)	

AV, atrioventricular; OFT, outflow tract.

differentiation from precardiac mesoderm. It is reasonable to speculate that such lack of reliance on one gene was likely gained as an evolutionary advantage. This could be the result of either (i) considerable genetic redundancy in precardiac myocyte differentiation, or (ii) an unsuspected diversity of precardiac myocyte lineages following independent genetic pathways. By this time in development, precardiac cells begin to express a variety of specific molecular markers, such as the transcription factors NKX2-5, MEF2, HAND1, HAND2, GATA4, TBX5, and ISL1 [11–17]. The region of splanchnic mesoderm expressing precardiac markers is also known as the "heart-forming field." It is larger than the region that will eventually contribute cells to the heart tube [18]. In rodent embryos, precardiac mesodermal cells exhibit spontaneous contractile activity, indicating a relatively advanced state of differentiation toward the cardiac myocyte lineage [19,20].

The precardiac mesodermal cell mass continues to migrate cephalad as a coalesced single unit, rather than as a collection of independent cells. Ultimately, they pass on either side of the prochordal plate to finally converge in the midline cranial to the intestinal portal. Following their convergence, the total premyocardial cell population forms a horseshoe-shaped crescent called the first, or primary, heart field. The cues that enable and promote movement of these cells are provided by a noncardiac tissue, the endoderm, as demonstrated by experimental removal of the endoderm and/or ectoderm [10]. The extracellular matrix molecule fibronectin may be one of the important components of the endodermal surface to which the precardiac cells are responding [21].

Precursors of the endocardium follow similar migratory pathways as the precardiac cells, but with important differences. Pre-endocardial cells and pre-endothelial cells are known as angioblasts. The endocardial angioblasts are first detectable in the splanchnic mesoderm. Mesodermal cells are induced to enter the angioblast lineage by signals such as transforming growth factor beta (TGFβ) 2–4 and vascular endothelial growth factor (VEGF) signaling from the endoderm [10]. Endocardial angioblasts migrate anteriorly and to the midline with the premyocardial cell mass, but they do so as individual cells.

At this point in cardiac organogenesis, the developing heart has not yet obtained its full complement of cell populations necessary for development. Based on early fate-mapping studies [22–24], the primary bilateral fields of precardiac mesoderm that form the early heart structure are collectively called the first heart field. This precardiac mesoderm was long considered the precursor tissue of the heart. Further studies then showed that growth of the heart tube, specifically at the arterial pole, depended on the addition of cardiac tissue from a secondary pool of progenitor cells [25]. It was not until the early twenty-first century that the nature of this additional cell population, called the second heart field, was finally elucidated. The combined studies of various laboratories [26–30] have provided significant

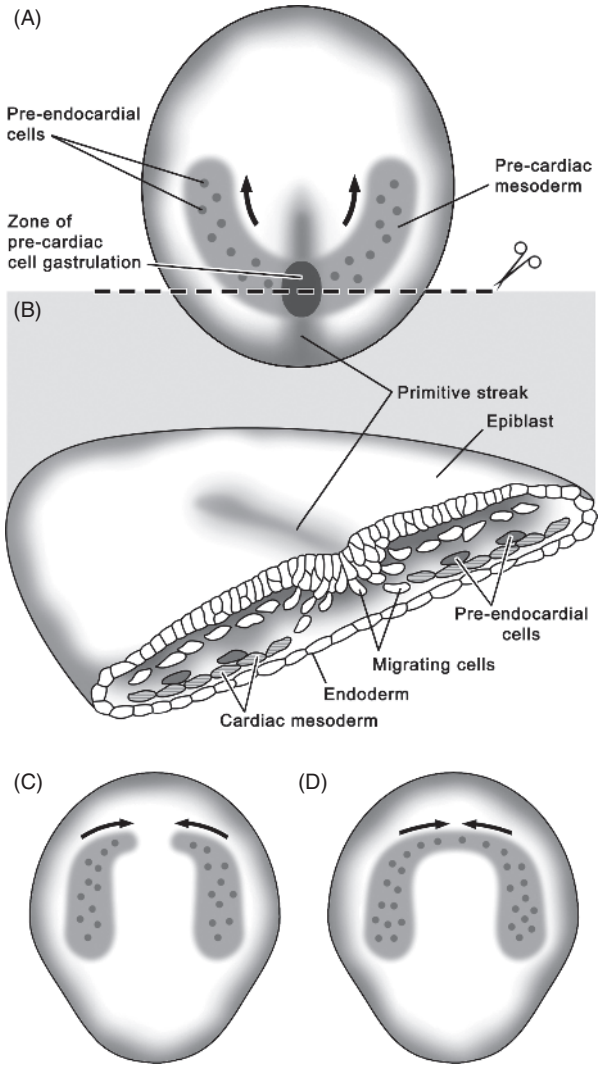

Figure 1.1 Simplified schema of gastrulation, precardiac cell migration, and formation of the heart-forming fields. (A) Cells destined to become cardiac cells migrate from the epiblast into the primitive streak through a broad region caudal to the most anterior portion of the primitive streak. The direction of migration of the gastrulated cells, as indicated by the arrows, is away from the midline and anteriorly on each side. (B) The embryo in cross-section at the level indicated by the dotted line in (A). The precardiac mesoderm forms an epithelial sheet closely associated with the endoderm. The pre-endocardial cells are scattered throughout the same region and can be distinguished immunohistochemically from the general precardiac mesoderm. (C, D) Two lateral precardiac mesoderm populations (also known as heart-forming fields) migrate cranially before turning toward the midline (C). They meet in the midline, as shown in (D), at a location immediately cranial to the anterior intestinal portal. *Source*: McQuinn T, Wessels A 2003 / With permission of Elsevier.

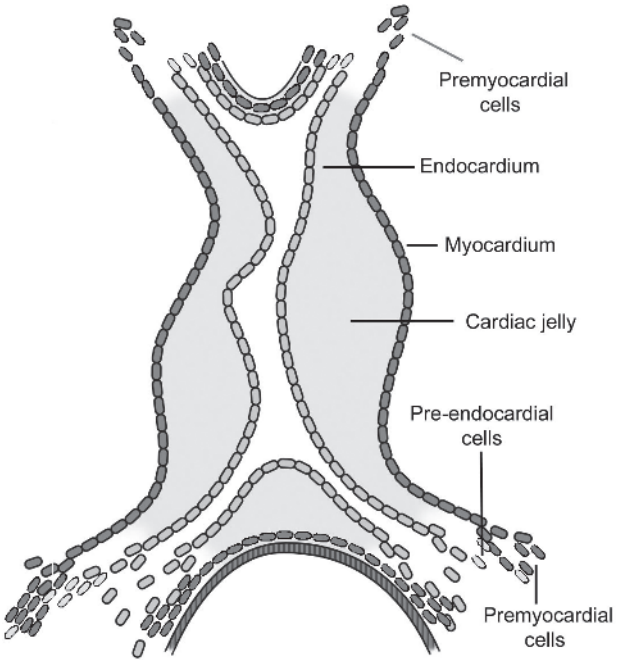

Figure 1.2 Formation of the heart tube is initiated by fusion of the bilateral precardiac mesoderm populations in the midline, resulting in formation of a myocardial tube surrounding an endothelial (endocardial) channel. The myocardial population of the cardiac tube at this stage consists of only the precursors of the future trabeculated portions of the left ventricle. Additional segments are added by ongoing migration of precardiac mesoderm into the heart tube. *Source*: Reproduced by permission from McQuinn T, Wessels A, in *Pediatric Cardiac Surgery*, 3rd ed. Philadelphia, PA: Mosby; 2003, pp. 1–24.

new insights into the importance of this additional population of cells regarding the elongation and growth of the heart tube, the outflow tract development, and the formation of a mature four-chambered heart (Figures 1.2 and 1.3) [3]. This second cardiogenic field of cells is located dorsal and caudal to the wall of the developing pericardial cavity, and lies contiguous with and medial to the primary cardiac crescent. Immunohistochemical, in situ hybridization, and cell fate-tracing techniques have demonstrated that the outflow tract primordia, the right ventricle, much of the muscular ventricular septum, and parts of the cardiac venous pole develop from the second heart field [29,31].

A developmentally distinct, third set of cells, the neural crest, contributes to the ultimate structure of the heart. These arise from the ectodermal neural tube. In vertebrate development, the neural crest is a transient structure originating in the most dorsal region of the neural tube [32,33]. The cells of the neural crest reside on the lateral

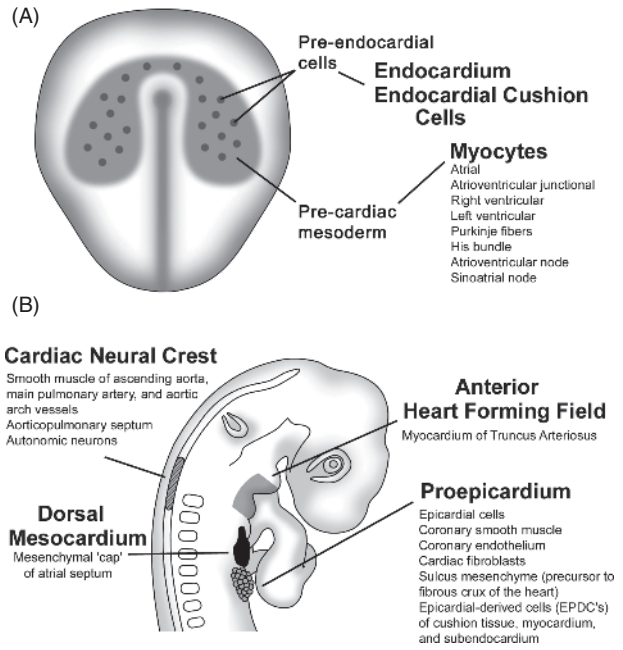

Figure 1.3 Normal heart development requires integration of cell populations from multiple sources. (A) Precardiac mesoderm gives rise to the endocardium and most cardiac cushion cells. Premyocardial cells give rise to the entire spectrum of cardiac myocyte phenotypes. The primary heart-forming field will give rise to most of the myocardium of the atria and left ventricle. The anterior heart-forming field will give rise to the myocardium of the outflow tract, right ventricle, and interventricular septum. (B) Multiple extracardiac embryonic tissues provide critical cell populations to normal cardiac development. These cell populations include cardiac neural crest cells as well as cells from the proepicardium and the dorsal mesocardium. *Source*: McQuinn T, Wessels A 2003 / With permission of Elsevier.

margins of the neural plate. These right- and left-sided populations are brought into apposition by the folding of the neural plate into the neural tube. The cells then disperse and migrate to multiple destinations. As with closure of the neural tube, this process is initiated cranially and extends caudally [9]. Specific regions of the neural crest seed cells to specific structures [34]. The region of the neural crest contributing to cardiac and fourth aortic arch morphogenesis is often referred to as the "cardiac" neural crest [9,32]. Cells from the cardiac neural crest migrate along the third, fourth, and sixth aortic arch vessels to reach the developing heart. These cells are necessary for aortopulmonary septation, outflow tract septation, and formation of the tunica media of the third, fourth, and sixth aortic arch vessels [32,35]. An additional population of neural crest cells differentiates into the entirety of the autonomic nervous system of the heart [36]. Other neural crest–derived structures in proximity to the developing heart and great vessels include the thymus, thyroid, and parathyroid glands [37,38].

Last, an epicardial layer of the heart develops from a histologically and functionally complex precursor tissue called the proepicardium, or proepicardial organ [39]. This is a transient cluster of cells that arise as a mesothelial outgrowth ventral and caudal to the developing heart. Soon after formation, the proepicardium migrates away from the body wall, with its cells extending over the surface of the heart to give rise to the epicardium. These cells eventually flatten over the surface of the myocardium, and develop morphologic characteristics compatible with primitive epithelial cells. The flattening process also causes cells to occupy a greater surface area, bringing adjacent clusters of cells into contact with each other until a continuous sheet results. A subset of the proepicardial cells invades the underlying heart tube and contributes to nonmyocardial mesenchymal cells such as fibroblasts and smooth muscle cells of the coronary arteries. In the mouse, where the process is well studied, these events occur in mid-gestation, although in the human they likely occur earlier [39,40]. Building on prior work, more recent studies suggest an important role for the epicardium in the diversification of cardiac lineages and potentially as a source of progenitor cell populations [41,42].

Formation of the Heart Tube and Early Segmentation

The initial cardiac crescent is located cranial to the prochordal plate. With closure of the neural tube, there is rapid cephalad growth of the central nervous system such that it extends over and around the developing heart. This leads to a cephalocaudal flexion of the embryo, bringing the cardiogenic plate ventral and caudal to the prochordal plate. At approximately the same time, the embryo folds in a transverse plane, bringing onto close apposition the lateral tubes of endothelial cells that were formed from early angioblasts. These tubes then fuse in a cephalocaudal direction to form the primitive single endocardial heart tube. Soon after the formation of the cardiac tube, the heartbeat is initiated and blood circulation can be observed. These events take place at embryonic day 8.5 in the mouse, and day 20 in the human. With the initiation of circulation, the heart becomes the first organ to adopt its essential mature function in the embryo. The heart tube at the time of its formation is connected to the foregut along its dorsal surface throughout its length by a structure called the dorsal mesocardium [43]. As cardiac looping proceeds, the dorsal mesocardium degenerates until it remains connected only at the atrial and arterial poles of the heart. The disintegration of the central portion of the dorsal mesocardium is a key event for looping to proceed

normally, while the arterial and venous attachments provide "anchors" for the looping heart tube. A dorsal mesenchymal protrusion, described centuries earlier as the "spina vestibule" or vestibular spine [44], extends into the atrial walls through the rightward margin of the persisting dorsal mesocardium, and is likely a derivative of the second heart field [28,45]. An important pathway for cellular migration, it contributes to atrioventricular (AV) septation, and serves as a scaffold for entry to the atria of the developing pulmonary veins [46,47].

At the time the heartbeat is initiated, the heart tube primarily consists of cells from the first heart field that form the future left ventricular tissues [48,49]. Cells that form the outflow tract, the right ventricle [25], the AV junction [48], the atria, and the systemic venous sinus, also known as the sinus venosus, are added to the heart as looping proceeds [50]. In prelooping and early looping stages the primitive heart tube consists of circumferential sheets of myocardial cells 2–3 layers thick surrounding the inner endothelial tube. These layers are separated by an acellular matrix–rich space known as the cardiac jelly. As looping continues, the future segments can be distinguished morphologically by their position in the heart tube, and by structural features, such as the concentration of cardiac jelly in the AV canal and outflow segments. Segments can be distinguished physiologically by measurement of the differences in their velocity of muscular contraction and relaxation, their rates of spontaneous pacemaker activity, and the speed of electrical impulse conduction [51,52].

Segmental differentiation creates the physiologic competence of the embryonic heart [53]. Unidirectional antegrade blood flow is maintained by organization of the heart tube into alternating regions of rapid and slow contractile properties [54]. The atrium has the fastest rate of spontaneous contractility, and is the site of pacemaker activity. A wave of depolarization spreads from myocyte to myocyte from the atrium to the outflow tract, but the velocity of conduction is not uniform throughout the length of the heart tube. Atrial conduction is rapid, AV conduction is slow, ventricular conduction is rapid, and outlet conduction is slow. The zones of rapid conduction show rapid contraction–relaxation mechanical properties, while the slow zones of conduction demonstrate slow, sustained contractions. The result is a forceful contraction of the atria, followed by a sphincter-like contraction of the AV junction. Prior to maturation of AV valves, these contractions prevent the retrograde flow of blood during the forceful ventricular ejection phase. The cardiac cycle of the heart tube is completed by a sphincter-like contraction of the outflow tract. Prior to maturation of the arterial valves, this area similarly prevents retrograde blood flow from the aortic arches.

In addition to these functional differences, the cardiac segments can be distinguished by unique patterns of gene expression. While data suggest that the final determination of lineage fate likely occurs in the precardiac mesoderm [24,55], the timing and nature of the mechanisms are incompletely understood. Perhaps the best-studied molecular determinants of the dorsal–ventral axis in the gastrulating embryo are retinoids [56,57]. Retinoids are products of vitamin A metabolism. Manipulation of retinoid signaling pathways results in significant abnormalities in axial patterning in general and cardiac development [58–60]. Abnormal development of the atrial segments and systemic venous tributaries is observed in conditions of retinoid deficiency [61–63]. Excess retinoids create malformations often involving the outflow tract [64,65], and result in ventricular expression of several genes that are normally largely restricted to the atria at these stages of development [66–68]. The spatial and temporal patterns of retinoid signaling in early cardiac development are highly correlated with the presence of retinaldehyde dehydrogenase 2 (RALDH2), a key enzyme in the retinol (vitamin A) to retinoic acid conversion pathway [69–71]. Retinoid signaling pathways are clearly key mechanisms of segmental differentiation within the heart, but it is clear that other components intersect and augment this pathway.

Looping

With the addition of cardiac tissue to the arterial and venous poles, the heart tube elongates and begins to bend. Looping becomes more complex as morphogenesis proceeds. The cranial portion of the tube bends caudally and to the right, while the caudal portion bends cranially to the left. The net outcome of this looping results in the left ventricle being positioned inferior and anterior to the atrium, while the right ventricle is slightly anterior and to the right of the left ventricle. The mechanisms regulating bending of the heart tube are incompletely understood. Interestingly, many looping movements are intrinsic to the heart, and can be observed even if the heart is isolated from the embryo, with or without beating [72,73]. Evidence suggests that deformation of the linear heart tube into a looped structure may be a result of mechanical force [74]. Additionally, bending may simply be the result of asymmetric changes in cell shape. Alternatively, myocytes may replicate faster in the larger curvature of the loop, and more slowly in the lesser curvature [75,76]. Additional studies suggest that asymmetric mechanical tension in the developing heart can induce bending. Experimentally, inhibitors of actin polymerization and cytoskeletal rearrangements can either abolish looping or reverse its direction, according to whether they have been universally or selectively applied [77].

Regardless of the regulatory mechanisms governing the process, bending of the heart tube confirms that the left and right sides of the embryo will not be morphologic mirror images. Although initial evidence of laterality is found in the atrioventricular canal prior to looping, cardiac development is inextricably linked to correct establishment of the three body axes [78,79]. All vertebrate, and most invertebrate, body plans demonstrate fundamental asymmetry about the three body axes: cranial–caudal (C–C), dorsal–ventral (D–V), and left–right (L–R). At the molecular level, the axes are determined by asymmetric propagation of signaling events early in development. The process of L–R axis determination as it governs heart development is broadly conceptualized as requiring three steps, which involve the initiation, elaboration, and interpretation of the sidedness signals [80,81]. The first step requires initiation of polarity along the C–C, D–V, and L–R axes. The second step is an elaboration and amplification of the initial L–R asymmetry. As is true in general for developmental processes, most of the molecules controlling the L–R signaling process in mice have recognized homologues in multiple species. Emphasizing the necessity to carefully examine multiple models, some of the molecules required for *left*-sidedness in mice are determinants of *right*-sidedness in birds [82]. The third step of axis determination is the interpretation of the asymmetric signals elaborated in the second step by the cells and tissues of developing organs. Paired structures develop as two fates, either as mirror symmetries, such as the limbs and parietal body parts, or as paired but unequal structures, such as the lungs, abdominal organs, and atrial appendages. These decisions are governed by both the signal delivered to the organ primordia as well as the reaction of the organ primordia to the signal. Such L–R axis determination in cardiac development is complex and several genetic models of abnormal cardiac looping have been described in mice [83]. Some models reflect an abnormal direction of looping, while others also show misalignment of cardiac segments. Mouse models of globally randomized arrangement of the organs [84,85], global mirror imagery [86], defects affecting different embryonic organs [87], and defects with preferential bilateral right- or left-sidedness resulting in isomerism have all been described [88]. Some genes implicated in the mouse models are likely also important in human isomerism [89]. There is often an increased incidence of abnormal ventriculo-arterial connections in mouse models of abnormal L–R axis determination [73], suggesting an element of L–R signaling in normal development of the outflow tract.

Looping determines not only the sidedness of the heart, but, importantly, the correct relationship of the segments of the heart to each other. After looping, it is possible to recognize inner and outer curvatures relative to the ventricular components of the heart tube (Figure 1.4). By nature of the tight angulation of the inner curvature, the primitive atrial, AV, ventricular, and outflow segments are near each other for the remainder of cardiac development. The inner curvature of the heart is arguably the most critical, complex, and dynamic site in normal cardiac development: it is the region where the right ventricle acquires its inflow, and the left ventricle acquires its outflow. On the luminal surface of the heart, the fold in the heart at the inner curvature results in a small muscular ridge inside the heart between the AV junction and the outflow tract called the ventriculo-infundibular flange, or ridge. Other key landmarks are the two major endocardial cushions of the AV junction, and the two comparable endocardial cushions extending through the outflow tract. The inferior endocardial cushion is attached to the dorsal AV myocardium, while the superior cushion is attached to the ventral AV myocardium. Then, small right and left lateral AV endocardial cushions arise. The two outflow cushions in the outflow tract form extended spiral ridges, initially extending from the pericardial margins cranially to the body of the right ventricle caudally. The endocardial ridge ending in the anterior right ventricle is called the septal endocardial ridge. The endocardial ridge terminating posteriorly in the right ventricle is called the parietal endocardial ridge. The parietal endocardial ridge contacts the right lateral endocardial cushion, which itself will become continuous with the superior endocardial cushion. The septal endocardial ridge contacts the inferior endocardial cushion. As the atrioventricular endocardial cushions fuse during normal septation, together with the mesenchymal cap of the primary atrial septum and the dorsal mesenchymal protrusion, they create the central AV mesenchymal complex. This process continues, via the outflow cushions, to the distal extent of the myocardial outflow tract. The distal margin of the myocardial outflow tract, however, itself regresses proximally during development. The distal outflow tract is then separated by growth of an additional protrusion from the dorsal wall of the aortic sac. It is this protrusion that forms the embryonic aortopulmonary septum. At the most basic level, the process of fusion of mesenchymal tissues can be thought of as a tissue zipper that septates the heart [45]. With respect to human congenital heart disease, this is a critical series of events. Disease phenotypes such as transposition of the great arteries, common arterial trunk, and several tetralogy variants all derive from aberrations of these normal developmental steps. This developmental insight has informed the genetics of congenital heart disease. Similar, yet discrete, anatomic phenotypes may have mechanistically less to do with each other than more disparate anatomic phenotypes derived from common developmental mechanisms.

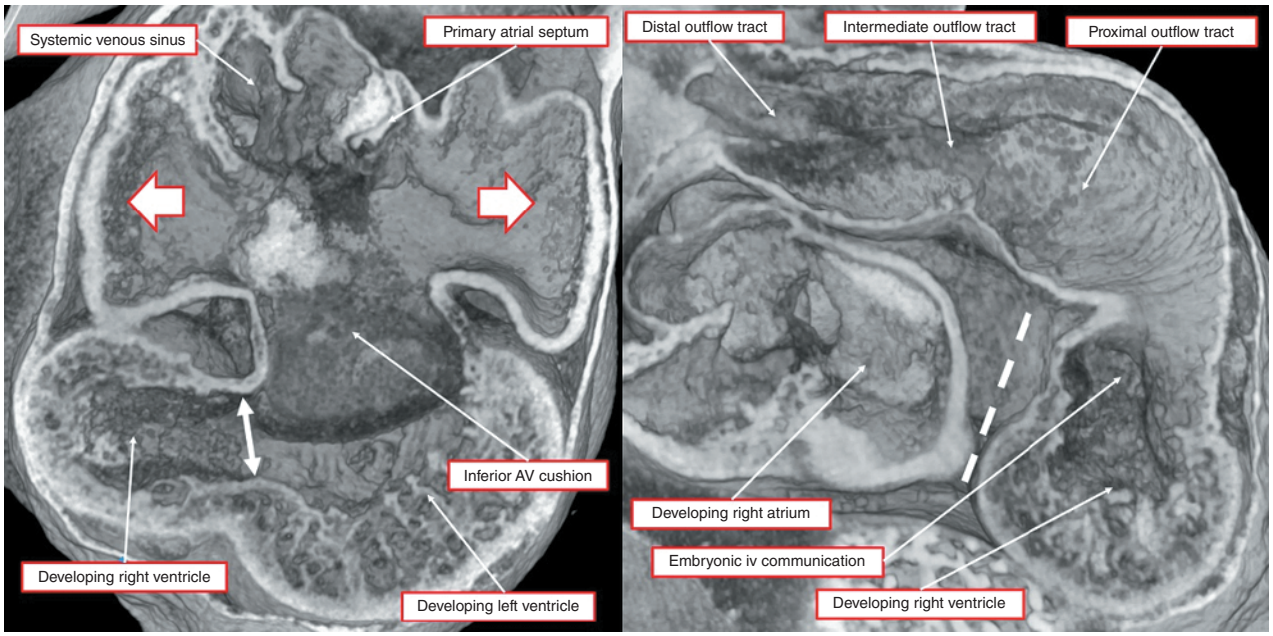

Figure 1.4 The images are from an episcopic dataset prepared from a human embryo at Carnegie Stage 14, equivalent to day 11 of development in the mouse (E10.5). The left-hand panel shows a section through the atrial and ventricular components of the developing heart after looping. The systemic venous sinus has been transferred to open to the right side of the atrial component of the primary heart tube, and the atrial appendages are beginning to balloon to right and left (white arrows with red borders). The primary atrial septum has begun to grow from the atrial roof toward the atrioventricular canal. The ventricular component of the heart tube has looped to the left, and the apical ventricular components are beginning to provide the primordiums of the developing right and left ventricles. The double-headed white arrow shows the embryonic interventricular communication, which is roofed by the inner heart curvature, since at this stage the atrioventricular canal is supported exclusively by the developing left ventricle. The right-hand panel shows how the developing right ventricle supports the outflow tract, which has proximal, intermediate, and distal components. Cushions extend through the intermediate and proximal components, but the distal part has a wide-open lumen. Note that, at this early stage, the developing right atrium has no direct connection with the developing right ventricle, with the dashed white line showing the extensive right atrioventricular groove. AV, atrioventricular, iv, interventricular.

The right AV junction is formed by rightward expansion of the AV junction, while at the same time the midline superior and inferior endocardial cushions are approaching each other in the center of the lumen of the AV junction. The outflow tract endocardial cushions, although unfused, define distinct proximal aortic and pulmonary channels. The aortic channel moves leftward and ventral of its original position. Because of the combined rightward expansion of the AV orifice, and the leftward movement of the aortic outflow tract, the acute angle of the inner curvature now defines the region where the AV junction and the outflow tract are in continuity. These same morphogenetic movements result in rotation of the proximal outflow cushions to a plane that is more closely parallel to that of the growing muscular ventricular septum.

Following initial heart assembly, the various segments of the heart continue to develop simultaneously, yet often at different rates. For the rest of the chapter, these events will be presented from the standpoint of the final cardiac segments formed, rather than in the temporal order in which they develop.

Development of the Inflow (Venous) Pole of the Heart

Systemic Venous Inflow

The embryonic systemic venous tributaries are formed by a process of vasculogenesis (Figures 1.5 and 1.6). Initially there are three bilaterally symmetric venous drainages: (i) the vitelline or omphalomesenteric, (ii) umbilical, and (iii) cardinal venous systems [90,91]. The vitelline veins drain the embryonic gastrointestinal tract and gut derivatives. The umbilical veins bring oxygenated blood from the placenta to the heart. The cardinal venous system returns blood from the embryonic head, neck, and body wall. All three of these drainage systems enter the systemic venous component of the primitive heart tube, known as the sinus venosus. The final, adult pattern of

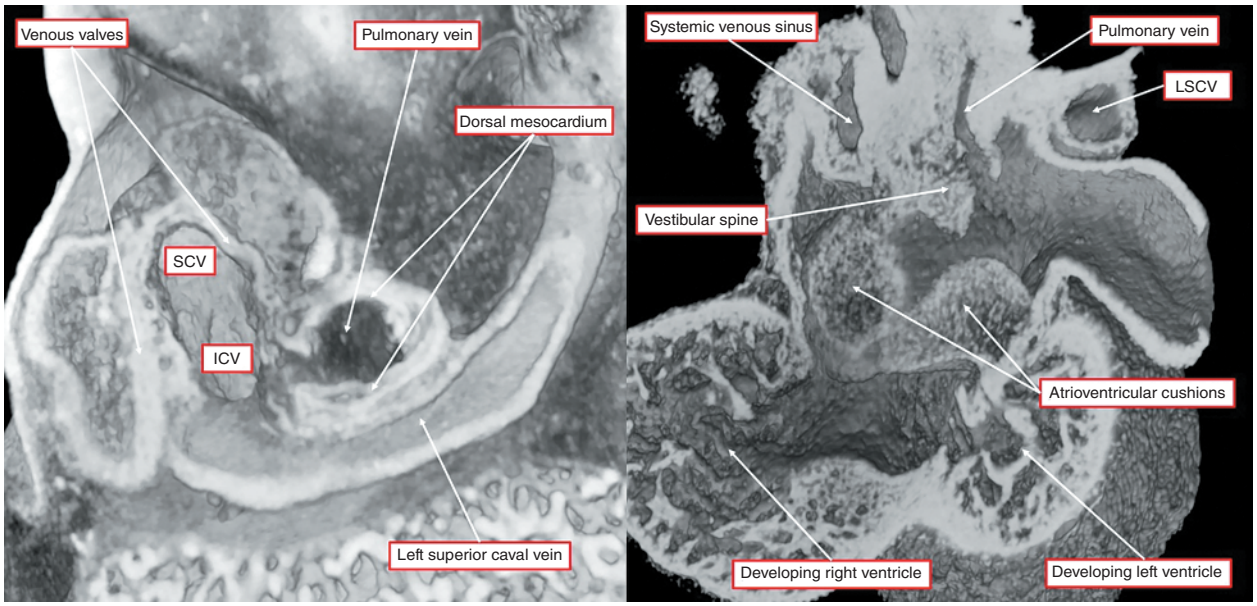

Figure 1.5 The images show how the systemic and pulmonary veins gain their connections to the right and left sides of the initial common atrial component of the primary heart tube. The left-hand panel is a dorsal coronal section through the venous pole of the human embryo shown in Figure 1.1, at Carnegie Stage 14. The left superior caval vein is cut in its long axis as it extends through the dorsal left atrioventricular groove. It opens to the right side of the atrial components, along with the right-sided superior caval vein and the inferior caval vein (SCV, ICV), with the boundaries of the systemic venous sinus marked by the venous valves. The section is cut through the connection of the atrial component of the heart tube to the pharyngeal mesenchyme, which is the dorsal mesocardium. The orifice of the pulmonary vein is visible in the center of the connection. The right-hand panel is from an episcopic dataset prepared from a mouse embryo sacrificed at E11.5. It shows how the rightward margin of the dorsal mesocardium has proliferated to become the vestibular spine, with its growth serving to commit the pulmonary vein to the cavity of the developing left atrium. Note that the left superior caval vein (LSCV) has its own discrete walls as it runs behind the cavity of the left atrium to enter the atrioventricular groove.

venous drainage is established through complex patterns of regression, remodeling, and replacement of these embryonic venous systems and their connections to the definitive right atrium [92].

Initially, the systemic venous component is bilaterally symmetric, with no anatomic boundaries initially present between it and the atrial component of the heart tube. As development progresses, the junction of tributaries and atrium shifts toward the right, perhaps influenced from blood shunting left to right. Normally, the connections of the left-sided cardinal, vitelline, and umbilical venous systems with the left horn of the systemic venous component regress. This results in the coronary sinus remaining as the primary structural derivative of the left horn of the systemic venous sinus in the normal fetal and postnatal heart. Failure of regression of the connections with the left venous horn results in persistence of the left superior caval vein. The right horn of the systemic venous sinus normally accommodates the entirety of the systemic venous drainage, except the portion from the heart, which is returned via the coronary sinus. A portion of this right horn is incorporated into the right atrium to form its smooth systemic venous portion between the orifices of the caval veins. At the same time, it is possible to recognize valves at the boundaries between the atrial and venous components. The inferior portion of the rightward venous valves becomes the Eustachian and Thebesian valves. They are related to the orifices of the inferior caval vein and the coronary sinus, respectively. During these processes, the umbilical and vitelline plexuses remodel and regress. Remodeling of the umbilical system produces the venous duct, or ductus venosus, which retains its connection with the cardinal system so as to connect to the developing inferior caval vein, thus bypassing liver sinusoids derived from the vitelline system. After birth, the venous duct becomes ligamentous. Hence, no derivatives of the embryonic umbilical venous drainage connect to the heart or persist in postnatal life.

The cardinal venous system initially consists of bilateral anterior cardinal veins, which drain the cephalic portion of the embryo, and bilateral posterior cardinal veins, which drain the caudal portion of the embryo. Fusion of the cardinal veins at the level of the systemic venous sinus forms the sinus horns, or common cardinal veins. The left anterior cardinal vein eventually loses its connection with the left sinus horn, but a small remnant on the

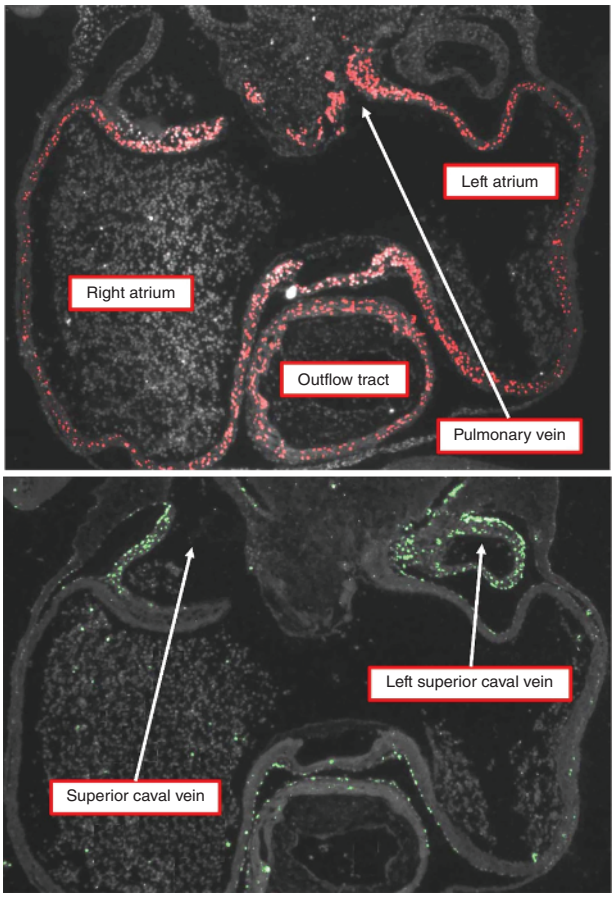

Figure 1.6 The images are from a serially sectioned human embryo at Carnegie Stage 14. The upper section has been incubated with an antibody to NKX 2.5, which marks the myocardium of the atrial walls and the proximal part of the outflow tract. The opening of the pulmonary vein can be seen between the margins of the dorsal mesocardium. The antibody does not, however, mark the walls of the systemic venous tributaries. As is shown in the lower panel, which is an adjacent serial section but incubated with an antibody to TBX18, the venous myocardium is positive to the second antibody, which does not mark the myocardium of either the atrial walls or the outflow tract. The findings support the notion that the pulmonary venous myocardium is not derived from the systemic venous component of the developing heart. Reproduced with the permission of Aleksandr Sizarov.

surface of the heart normally persists as a passage of coronary venous blood to the coronary sinus, and is known as the oblique vein of the left atrium. Another portion of the left anterior cardinal vein persists as the left internal jugular vein. As the left anterior cardinal vein loses connection with the heart, it becomes connected to the right anterior cardinal vein via the intercardinal anastomosis that forms between the thyroid vein and the thymic vein. This connection persists as the left brachiocephalic vein. The portion of the right anterior cardinal vein between the right atrium and the drainage of the left anterior cardinal vein proximally, via the intercardinal anastomosis, becomes the normal right superior caval vein.

The posterior cardinal veins originally drain the body wall, gonadal, and renal structures. Their function in venous drainage of the body wall is supplanted by the supracardinal venous plexus. The gonadal and renal venous drainage is captured by the subcardinal venous plexus and the lower extremities are drained by the sacrocardinal veins. The various venous beds contribute the segments that form the definitive inferior caval vein to the level of the persisting segment derived from the right vitelline vein, which then connects to the right atrium. The supracardinal venous system persists as the azygous and hemiazygous veins.

In both the supracardinal and subcardinal venous systems, the initial vascular structures are bilaterally symmetric. The left-sided channels subsequently regress, underscoring the typical right-sided location of the inferior caval vein. The frequency of venous drainage abnormalities in human and animal models of altered L–R axis differentiation [88] strongly suggest that the mechanisms of venous morphogenesis depend upon appropriate left–right signaling.

Pulmonary Venous Inflow

The earliest evidence of the formation of the common pulmonary vein in the embryo is the presence of a strand of endothelial cells in the pharyngeal mesenchyme, pointing to the dorsal mesocardium. This endothelial strand forms a lumen, and initially is a midline structure. As the vestibular spine is developing and projecting into the atrial cavity on the right side of the primitive pulmonary vein, the relative position of the common pulmonary vein changes as it occupies a position to the left of center [93]. The continued development of the vestibular spine [44], the myocardialization of this mesenchyme, and the concomitant growth of the primary atrial septum eventually result in the normal connection of the pulmonary vein to the left atrium [94]. The development of the pulmonary vein does not result from an outgrowth of the atrial wall. During this process, the lungs themselves develop from the foregut, with venous elements from the lungs bilaterally fusing with the common pulmonary vein. Abnormal formation of the common pulmonary vein results in persistence of initial connections of the pulmonary plexuses to the cardinal veins, resulting in various forms of anomalous pulmonary venous return. Initially, the walls of the pulmonary veins are not muscular. As development proceeds, myocardial sleeves are formed around these veins [95]. Semaphorin signaling

has been linked to defects in this process [96]. As pulmonary myocardium is a frequent site initiating atrial fibrillation, a clear understanding of the molecular and genetic mechanisms that direct these events is important.

Atrial Septation

The major steps in cardiac septation occur between the fourth and fifth weeks of human gestation. Proper atrial septation requires two important processes. The first is differential proliferation of tissues: for example, the folds often described as the "septum secundum." The second process is active proliferation of cells that approach each other and fuse, resulting in septation of the chamber: for example, growth of the primary septum and the endocardial cushions. Atrial septation and connection of the common pulmonary vein to the left atrium are closely related events in the normal heart [94]. Experiments in mouse, chick, and human embryos highlight the importance of the dorsal mesocardium to these events [44]. Unlike other mesenchymal tissues in the AV junction, the vestibular spine, which grows through the rightward margin of the dorsal mesocardium, is not derived from an epithelial-to-mesenchymal transformation, but rather is a derivative of the second heart field [28,45]. The vestibular spine extends into the atrial cavity, becoming contiguous with the mesenchymal cap on the leading edge of the primary atrial septum. Together with the superior and inferior atrioventricular cushions, the vestibular spine and the cap eventually fuse to form the atrioventricular mesenchymal complex. This process is essential for normal atrioventricular septation (Figure 1.6). Recent work suggests that perturbation of the development of the protrusion might be one of the contributing mechanisms in the pathogenesis of atrioventricular septal defects [97].

Based on the expression of several molecular markers that distinguish left and right atrial myocardium, the primary atrial septum is derived from left atrial myocardium. Growth of the septum occurs by lengthening of its myocardial portion. As described above, the cap on the leading edge of the septum primum is mesenchymal, the cells being derived by endothelial-to-mesenchymal transformation like that seen in the endocardial cushions [93]. As growth of the septum proceeds, it brings the mesenchymal cap, as well as the vestibular spine, into contact with the AV endocardial cushions (Figure 1.7).

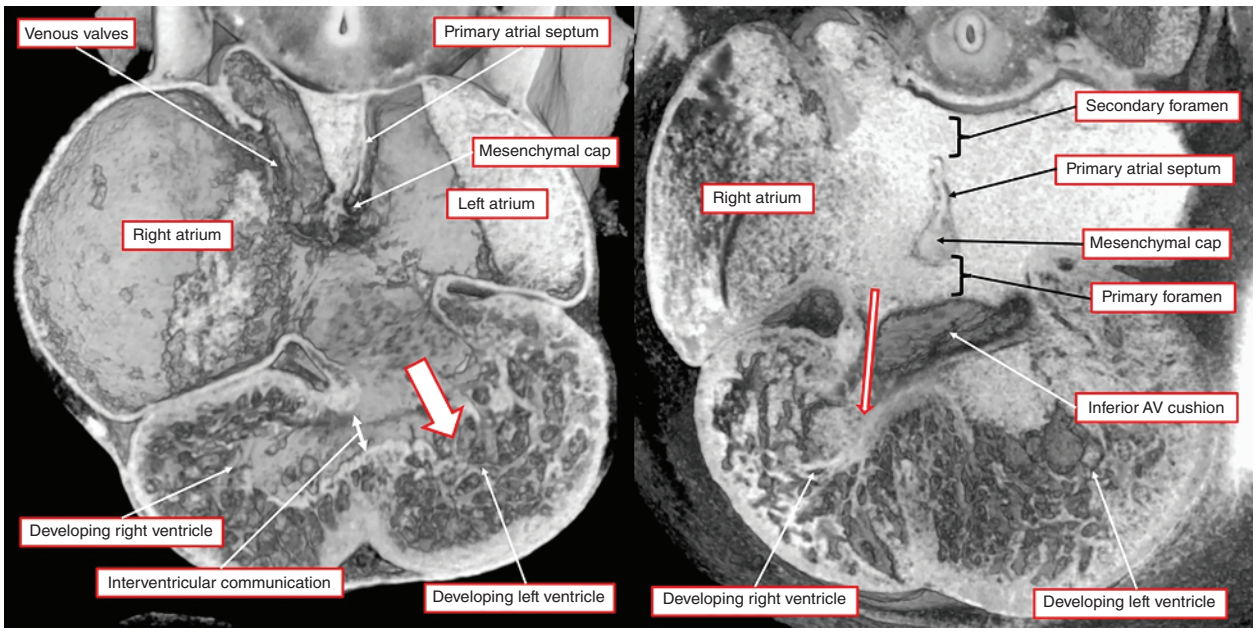

Figure 1.7 The images are "four-chamber" sections through episcopic datasets prepared from human embryos at Carnegie Stage (CS) 14 (left-hand panel) and CS 16 (right-hand panel). They show how expansion of the right atrioventricular (AV) junction brings the cavity of the right atrium into direct continuity with that of the developing right ventricle. Initially, as shown in the left-hand panel, the AV canal opens exclusively to the developing left ventricle (white arrow with red borders). The blood then enters the developing right ventricle through the embryonic interventricular communication. Note the growth of the primary atrial septum toward the AV canal, with a mesenchymal cap on its leading edge. By CS 16 (right-hand panel), the primary septum has broken away from the atrial roof, producing the secondary interatrial foramen. The primary atrial foramen is now bounded by the mesenchymal cap carried on the primary atrial septum and the cranial margin of the inferior AV cushion. Note that, by virtue of the expansion of the right AV junction, the cavity of the right atrium is now in direct communication with that of the developing right ventricle (white arrow with red borders).

The primary interatrial foramen is closed by fusion of these mesenchymal tissues as they form the AV mesenchymal complex [45]. Knowledge of this process is critical to understanding the pathogenesis of deficient atrioventricular septation, as well as understanding the tissue relationships relevant to its repair.

Before closure of the primary interatrial foramen, the foramen secundum appears at the cranial margin of the septum primum. In humans, this process is initiated by the appearance of small fenestrations that increase in number and size until they coalesce into a definitive foramen [94]. The "septum secundum" then forms as an infolding of the atrial roof in the intervalvar space between the left venous valve of the systemic venous sinus and the primary atrial septum. The infolding also marks the site of the boundary between left and right atrial tissues.

Atrioventricular Valvar Formation

An important step in the formation of both AV and arterial valves is the formation of endocardial cushions. When the heart tube initially forms, the myocardial and endocardial cell layers are separated by an acellular substance traditionally called "cardiac jelly" [98]. As it lies between the juxtaposed myocardial and endocardial epithelia, cardiac jelly is a type of basement membrane that contains traditional basement membrane proteins. Cardiac jelly condenses into opposing swellings at the outflow and AV regions of the early, looped heart. The resulting endocardial cushions function in combination with the specialized contractile properties of the AV junction and outflow tract myocardium to limit reversal of blood flow [99]. The AV endocardial cushions also function as the substrate for the formation of the mesenchymal tissues of the crux of the heart, including the AV valves and central fibrous body [100]. Endocardial cushions of the embryonic outflow tract participate in the formation of the arterial valves and free-standing subpulmonary infundibular sleeve [101]. During morphogenesis of the endocardial cushions, the mesenchymal cell population that populates the original acellular cardiac jelly is derived from two sources: (i) the endothelial cells of the heart, and (ii) a population of epicardially derived cells that migrate into the AV cushions [93,102]. Interestingly, this cell population does not populate the outflow tract cushions, which are populated by cells derived from the neural crest [103].

Endothelial invasion of the cardiac jelly results in a true transdifferentiation of cell phenotype, from a cell within a typical epithelium to an independently migratory, fibroblast-like mesenchymal cell [104,105]. This process has been compared to cellular changes during malignant transformation. It is at least partially under the control of TGFβ-mediated signaling processes [106,107]. Only endocardium from the outflow tract and AV cushions is competent to undergo this transition, and only outflow tract and AV junction myocardium can induce transformation [106,108]. Not all the endocardial cells of the AV and outflow regions participate in these changes. As migration proceeds, residual endocardial cell populations undergo divisions to replenish their numbers. The mesenchymal cells also replicate actively to populate the cushions [104].

There are four endocardial cushions at the AV junction. The superior and inferior cushions are the first to appear, and are the most prominent endocardial cushion masses. There are also important contributions from the lateral endocardial cushions that are visible only after Carnegie Stage 17, representing approximately 42 days of development [100,109]. The left lateral cushion contributes to the mural leaflet of the mitral valve. The right lateral cushion, which becomes continuous anteriorly with the septal endocardial cushion of the outflow tract, contributes to the formation of the anterosuperior and inferior leaflets of the tricuspid valve.

Atrioventricular valvar leaflets are formed by separation of endocardial cushion tissue and myocardium from the ventricular walls via a poorly understood process of maturation that includes delamination [109–111]. Other simultaneous events influence normal valvar morphogenesis, and may contribute to the delamination process. These include incorporation of ventricular trabeculations to form the papillary muscles, and apical expansion of the ventricular cavity. At the time of delamination, the atrial surfaces of the valvar leaflets are composed of endocardial cushion tissue, while the ventricular surfaces are primarily myocardial. The myocardial layer provides continuity with the evolving subvalvar tension apparatus. As leaflet morphogenesis proceeds, the myocardial component is eliminated by poorly understood processes.

Initially, the ends of the AV leaflets are connected to the compact ventricular myocardium either directly or via the compacting trabeculations. As development proceeds, papillary muscles mature by two mechanisms. First, initially independent, pre-existing trabeculations coalesce to form papillary muscles. Second, myocardium delaminates into myocardial structures and joins with trabeculations to form papillary muscles [111]. The surgically challenging group of patients with parachute mitral valve derivatives are likely due to deficiencies in these processes. Tricuspid valve papillary muscles develop independently from each other and via distinct mechanisms. The anterior papillary muscle of the tricuspid valve in humans derives from an early coalescence of trabeculations detectable at 6 weeks' gestation. The papillary muscles supporting the septal leaflet are the product of delamination during the 10–12 weeks' gestation, while

the inferior papillary muscle complex is still a relatively indistinct structure at 12 weeks' gestation [110]. The tendinous cords are formed by progressive fenestration and fibrous differentiation of trabeculations and the initially solid individual valvar leaflets [109,112].

Atrioventricular valvar morphogenesis is one of the most prolonged aspects of human cardiac development. Recognizable elements of the tricuspid valve begin formation at 5–6 weeks' gestation. Now the AV endocardial cushions are actively reconfiguring, with fusion of the superior and right lateral AV endocardial cushions to each other. Simultaneously, the proximal outflow cushions are also completing their fusion. The fused outflow cushions then fuse with the crest of the muscular ventricular septum as the aortic root is transferred to the left ventricle. The surface of the cushions then muscularizes to form the supraventricular crest, with the core of the fused cushion mass attenuating to give rise to the fibroadipose tissues that interpose between the free-standing subpulmonary infundibulum and the aortic root. At this point, the tricuspid valvar leaflets are still very primitive in appearance and not yet freely mobile (Figure 1.8). The inferior leaflet is fully delaminated by the end of 8 weeks' gestation, the anterosuperior leaflet by 11 weeks, and the septal leaflet in week 12. The zone of apposition separating the anterosuperior and septal leaflets is not complete until the septal leaflet is fully delaminated [100].

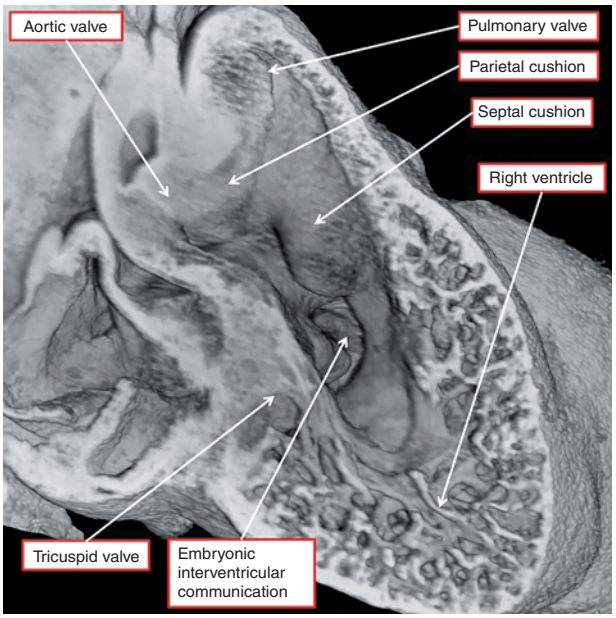

Figure 1.8 The image shows the developing right ventricle as seen having removed its parietal wall in a mouse embryo sacrificed early during E12.5. At this stage, the intermediate part of the outflow tract has separated into the aortic and pulmonary roots, but both roots remain supported by the developing right ventricle. The proximal outflow cushions have yet to fuse completely. The blood from the left ventricle, therefore, continues to enter the aortic root through the embryonic interventricular communication. It is necessary to transfer the aortic root to the left ventricle before ventricular septation can be completed.

In development of the mitral valve, the superior and inferior AV cushions begin to fuse at 5 weeks' gestation. Even prior to the completion of their fusion, the trabeculations will evolve into the two papillary muscles supporting the ends of the solitary zone of apposition between the developing aortic and mural leaflets. It is the fused superior and inferior cushions that give rise to the aortic leaflet of the mitral valve. The left lateral cushion, the precursor to the mural leaflet, is visible by week 7 of gestation. Now initial delamination of the mitral valvar structures becomes detectable and continues until the week 10 of development. Between weeks 10 and 14 of development, myocardial elements of the leaflets are eliminated, papillary muscles achieve their adult appearance, and there is differentiation of the tendinous cords [109,111].

The electrophysiologic and physiologic properties of the junctional myocardium between the primitive atria and ventricles are critical to the preseptated heart. Myocardial continuity between the AV junctional myocardium, the atrial myocardium, and the ventricular myocardium must be interrupted for the development of the fibrous crux of the heart and correct function of the conduction system. This is accomplished by formation of a layer of fibrous insulation, the fibrous annulus or annulus fibrosis. Except for the penetrating bundle of His, the insulating tissues will completely interrupt and insulate myocardial continuity between the AV and the ventricular myocardium. The fibrous annulus forms by fusion of mesenchymal cell populations of the AV endocardial cushions with a mesenchymal cell population found in the AV grooves on the external surface of the looped heart. Atrioventricular groove mesenchyme cells are brought to the heart during the epicardial cell migration (Figure 1.9) [3]. Studies suggest that the mesenchyme of the AV groove actively invaginates into the endocardial cushions, although the mechanisms driving mesenchymal invagination are unknown [113]. Interruption of myocardial continuity begins at 52–60 days' gestation in the human heart, and is complete by the fourth month [100]. Failure to properly form the insulating tissues of the AV junction may underlie clinical pre-excitation syndromes. Interestingly, isolated myocytes have been identified bridging the fibrous insulation of normal neonatal hearts. These myocytes may represent remnants of the embryonic junctional myocardium, originally present between the mesenchyme of the AV groove and the endocardial cushion mesenchyme. The relationship between pre-excitation pathways in general and normal morphologic events requires further investigation [114,115].

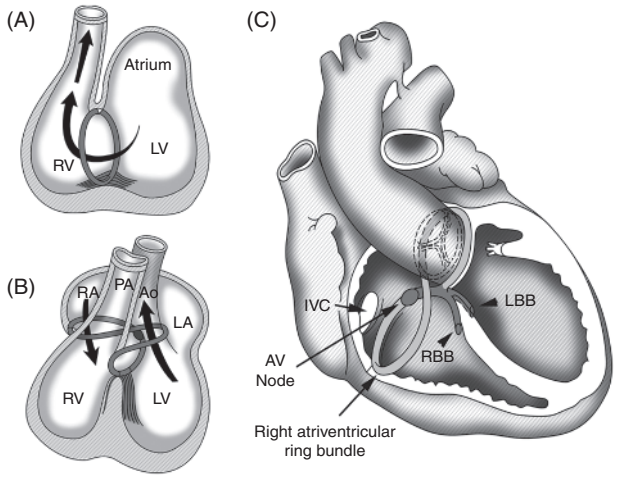

Figure 1.9 The development of the conduction system from a ring of myocardium detected in human embryonic heart by the expression of a carbohydrate epitope recognized by the antibodies Gln2, HNK1, and Leu7. (A) Human heart at roughly 5 weeks' gestation. A ring of myocardium surrounding the primary interventricular foramen is detected. Note that the superior aspect of the ring is at the junction of atrioventricular (AV) myocardium and ventricular myocardium. (B) In the human heart of roughly 7 weeks' development, convergence has resulted in expansion of the small segment of AV junctional myocardium to the right, accompanying the rightward expansion of the AV inlet. The leftward movement of the outlet results in the looping of the ring around the aortic root. As the muscular ventricular septum begins to grow, strands of ring tissue can be found extending from the major aspect of the ring on the crest of the septum down the septal walls toward the expanding apices of the right and left ventricles. (C) The mature cardiac conduction system of AV node, bundle of His, and right bundle branch (RBB) and left bundle branch (LBB) is derived from the primitive ring tissue. In addition, remnants of the primitive ring in the adult heart can be found on the atrial side of the tricuspid valve fibrous annulus as well as in retroaortic myocardium. Ao, aorta; IVC, inferior caval vein; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RV, right ventricle. *Source*: Adapted from Moorman AF et al. *Circ Res*. 1998;82:629–644; Wessels A et al. *Anat Rec*. 1992;232:97–111.

Atrioventricular valvar development may be tied to the process of ingrowth of the insulating tissues, as the hinge points of the definitive leaflets are normally found at the point of juncture between the endocardial cushion tissues and the invaginating tissue.

Ventricular Chamber Formation and Septation

Shortly after looping, the myocardial layers of the heart tube are only a few cells thick. After looping, the ventricular chambers enlarge caudally in a pouch-like fashion. The pouches are located on the greater curvature of the looped heart, and quickly develop a series of circumferential ridges on their internal surfaces (Figure 1.4) [3,112].

The myocytes of these primitive trabeculations differ from the subjacent compact myocardium in that the myocytes of the compact myocardium are actively proliferating, while the trabecular myocytes have withdrawn from the cell cycle and are not dividing. The "germinal layer" of compact myocardium provides increases in the numbers of ventricular wall myocytes, but the initial major increases in myocardial mass occur through increase in the trabecular component of the myocardium. The compact myocardium "feeds" cells into the ventricular junctions of the developing trabeculations, a relationship that persists throughout embryonic myocardial growth [3,112]. The primitive trabecular ridges become fenestrated and sponge-like as they expand. Trabeculations are hypothesized to assume several physiologic functions in the primitive heart. They may enhance contractile function of the ventricles [116]. The surface area of the endocardium is greatly increased by their presence. This increase may improve nutrient and gas exchange with the developing myocardium before the development of a true coronary vasculature [117]. The trabeculations have been shown to persist as the distal bundle branches and Purkinje fibers in the postnatal heart [54]. Trabeculations also help to direct the flow of blood in the preseptated heart [118]. Thus, the infrequent yet important echocardiographic finding of the presence of excessive trabeculations bears important implications [119].

The molecular signatures of the compact and trabecular myocardium are distinct. The compact myocardium can respond to signals that direct cell proliferation, and maintain the growth of the embryonic heart by adding new cardiomyocytes. These proliferative signals include proteins such as neuregulin that are secreted by the endocardial cells. This stimulates their cognate receptor proteins in the myocardium, thereby inducing proliferations [120,121]. Similarly, the epicardium may also secrete proteins such as insulin-like growth factor that can trigger cardiomyocyte proliferation [122,123]. The myocardial cells themselves are capable of initiating cell division directly, or indirectly via downstream signaling into the endocardial cells [124]. Recent evidence indicates that there may be extracardiac control of cardiomyocyte proliferation by molecules such as erythropoietin, which may be secreted in the developing liver [125]. Regardless of the source of these signals, the embryonic heart can add new cardiomyocytes to increase the extent of the trabeculations as well as the thickness of the compact layer of the myocardium. This capability is lost in postnatal life in mammals, such that any increase in heart size in the adult is a result of cardiomyocyte hypertrophy rather than the addition of new cells. What molecular changes are

responsible for this shift, and how they may be reversed, is a subject of active research in the field of cardiac regeneration. Data now suggest that the neonatal mouse is also capable of regenerating new cardiomyocytes, implying that the loss in regenerative capability is likely a postnatal event [126]. The increase in thickness of the compact layer of the embryonic myocardium may decrease diffusion of nutrients from the lumen. This correlates with and, via hypoxic signals, may accelerate the formation of a mature coronary vascular plexus. With the demand for increased surface area now diminished, a large portion of the trabeculations protrude into the myocardial wall, resulting in an even thicker ventricular wall. This process is more robust in the left ventricle, due to unknown mechanisms that correlate with the more densely trabeculated morphology of the right ventricle.

Ventricular septation is the process of closing the embryonic interventricular foramen while bringing the muscular ventricular septum into continuity with the muscularizing proximal outflow cushions (Figure 1.10). The embryonic interventricular foramen initially provides all the inflow to the right ventricle and the entire outflow for the left ventricle. It cannot be closed, therefore, until after the right ventricle has achieved its inlet and the left ventricle its outlet. These processes are achieved by remodeling the cavity of the initial primary heart tube. Only after this remodeling can the persisting interventricular foramen be closed by coordinated growth of the muscular interventricular septum and fusion of the endocardial cushions in the AV junction and the outflow tract.

Growth of the muscular interventricular septum is closely associated with dynamic changes in patterning of the ventricular myocardium [112]. In the mammalian heart, the primitive muscular septum appears to be the product of infolding of the compact myocardium produced by growth of the ventricular apices. The primitive muscular interventricular septum is initially a crescent-shaped structure that extends at its dorsal limit to the inferior endocardial cushion, and at its anterior limit to the superior endocardial cushion. Part of the process of closure of the primary interventricular foramen consists of expansion of the superior and inferior endocardial cushions toward each other, where they will make contact and fuse at approximately 6 weeks' gestation in the human. Further growth of the interventricular muscular septum results in fusion of the crest of the septum with the fused cushions. In humans, the AV membranous septum is the only nonmyocardial septal structure derived from endocardial cushion tissue. It is derived from

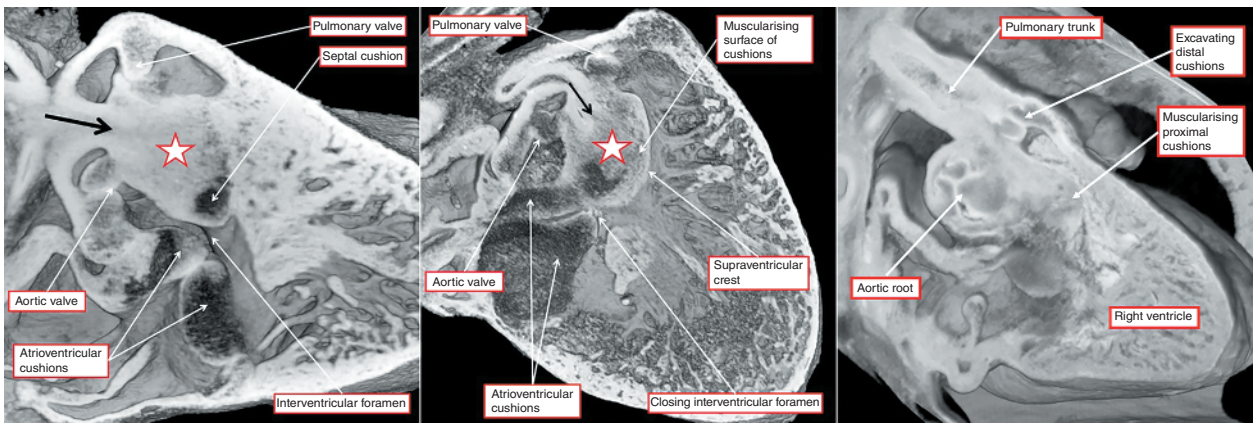

Figure 1.10 The images show the steps involved in the completion of ventricular septation. The left-hand panel is prepared from a mouse embryo sacrificed late during E12.5, while the right-hand panel is from an embryo sacrificed at E13.5. As can be seen in the left-hand panel, the proximal septal outflow cushion has fused with the crest of the muscular ventricular septum, committing the aortic root to the left ventricle. Tubercles growing from the ventricular surfaces of the atrioventricular cushions are now sealing off the interventricular communication. The middle panel shows how the tubercles come together with the margin of the outflow cushion to close the interventricular foramen. The tubercles, with ongoing development, will become the membranous part of the ventricular septum. The white star shows the core of the proximal outflow cushions, which will attenuate to produce the plane between the newly formed subpulmonary infundibulum, derived from the muscularized surface of the proximal cushions, and the aortic root. The black arrow shows the plane that has separated the aortic root from the pulmonary root. The trabeculations of the right ventricle coalesce to form the septomarginal trabeculation, or septal band. The right-hand panel shows a subcostal oblique section through the developing heart from a human embryo at Carnegie Stage 17. The distal parts of the outflow cushions, occupying the intermediate part of the outflow tract, are remodeling to produce the leaflets of the aortic and pulmonary valves. The distal part of the outflow tract itself has already separated to produce the intrapericardial components of the aorta and pulmonary trunk. Only the pulmonary trunk is seen in the section.

tubercles formed at the approximate site of final union between the muscular septum and the AV endocardial cushions [127].

Outflow Tract

In the early phases of looping, the outflow tract is short. Later it becomes elongated, with a distinct bend. The site of the bend is the primary external landmark dividing the distal component, sometimes referred to as truncus, from the proximal part, sometimes referred to as conus. The bend itself then achieves prominence, since the primordia of the arterial valves are formed at this site. The tract extends initially from the outlet of the developing right ventricle to the aortic sac. The distal boundary is marked by the reflections of the pericardium. Initially the entire tract is lined by cardiac jelly and has myocardial walls. As the jelly becomes converted into the outflow cushions, however, additional nonmyocardial tissues grow into the distal outflow tract from the second heart field. This produces shortening of the myocardial component, with regression of the distal myocardial border from the pericardial margins. The nonmyocardial component is then separated into aortic and pulmonary components by growth of an oblique protrusion from the dorsal wall of the aortic sac. Growing between the origins of the arteries of the fourth and sixth pharyngeal arches, this protrusion is the embryonic aortopulmonary septum. The persisting outflow tract with myocardial walls is separated by the outflow cushions into the ventricular outflow tracts and the arterial roots. At the base of the heart, the outflow cushions are continuous with the AV endocardial cushions. During remodeling of the proximal outflow tract, the mesenchymal continuity of the cushions will be retained between the mitral and aortic valves, but will be lost between the tricuspid and pulmonary valves [128,129].

The mechanisms that control outflow tract remodeling are increasingly understood. During early development, the outflow tract is positioned such that it receives blood only from the right ventricle. The addition of cells from the second heart field allows the elongation of this tract such that it is positioned medially over the ventricular septum [130–132]. For simplicity, three interrelated yet distinct processes are believed to govern the maturation of the outflow tract. The first of these is alignment, which ensures that the systemic and pulmonary outflow tracts align with the appropriate ventricle. The second is separation, resulting in the initially solitary cavity of the outflow tract becoming the separate aortic and pulmonary pathways. The third is rotation, such that the outflow from the pulmonary and systemic ventricles is joined to the appropriate arterial trunk. It is precisely these mechanisms that are of particular importance in understanding the pathogenesis of a large number of clinically challenging congenital heart disease phenotypes.

A deficiency of cells from the second heart field results in a shortened outflow tract, which cannot then be properly aligned with the ventricular septum. An extended discussion of these mechanisms can be found in Chapter 2. During their initial septation, the newly formed aortic and pulmonary roots remain connected to the right ventricle, resulting in double outlet right ventricle (DORV). A defect in outflow tract septation, but with normal outflow tract elongation and alignment, results in a medially positioned but undivided common arterial trunk. Finally, lack of proper rotation of the great vessels, or more likely a defect in the spiral septation of the aortic and pulmonary trunks, results in discordant ventriculoarterial connections, known as transposition of the great arteries. While the cellular components that participate in outflow tract morphogenesis are recognized, the molecular signals and cellular interactions that underlie normal morphogenesis have yet to be fully elucidated [133,134]. Several molecular pathways have been implicated in these processes, including retinoic acid signaling [135], TGFβ [106], and Notch pathway components [136].

Outflow Tract Septation

Separation of the intermediate and proximal parts of the outflow tract is a multistep process. Initially the developing endocardial cushions are "simple" structures consisting of cardiac jelly bounded by endocardium and myocardium. As in the AV cushions, a subset of endocardial cells transdifferentiates into mesenchymal cells that then invade the cardiac jelly. The cushions thus formed subsequently enlarge and are brought into apposition [99]. Fusion proceeds temporally from the union with the nonmyocardial distal outflow tract to the base of the heart, and results initially in the formation of a mesenchymal outflow septum. When the proximal cushions fuse, they complete the separation of the subaortic and subpulmonary outflow tracts. The right ventricular surface of the fused endocardial cushions is then replaced by myocardium, which is then transformed into the free-standing subpulmonary infundibular sleeve as the central core of the cushion mass attenuates. The newly formed myocardial component becomes the supraventricular crest, with no muscular outlet septum to be found postnatally.

The process of initial septation is dependent on the cells derived from the neural crest. These can be recognized as the so-called prongs that extend from the site of fusion of the aortopulmonary septum derived from the protrusion from the dorsal wall of the aortic sac and the distal margins of the outflow cushions themselves. The neural crest cells in the proximal cushions subsequently disappear, as they do in the intermediate part of the outflow tract.

It is the disappearance of these cells that creates the area of fibroadipose tissue that interposes between the arterial roots in the postnatal heart, and between the aortic root and the free-standing subpulmonary infundibular sleeve [137].

Development of the Arterial Valves

The arterial valves develop in the intermediate part of the outflow tract, at the junction between the aortopulmonary septum and the fused distal outflow cushions [129,138]. They are derived from the distal ends of the parietal and septal cushions, along with the intercalated cushions that form between the edges of the major cushions. Fusion of the cushions themselves occurs by contact, followed by disappearance of the endothelial cells at the site of contact. The site of fusion of the cushions with the protrusion from the dorsal wall of the aortic sac is marked by a whorl or knot-like structure. Prongs extend from the knot into the proximal cushions. In the past, these have been said to contribute to aortopulmonary septation. The so-called prongs, however, do not become visible until after the intrapericardial arterial trunks are separated one from the other. The prongs are involved with initial separation of the proximal outflow tract, which then becomes the outlets for the right and left ventricles.

Development of the arterial valvar leaflets begins shortly after septation of the intermediate part of the outflow tract. In mice, the initial process is excavation of tissue corresponding to the future leaflets from the arterial surface of the distal and intercalated cushions [139]. Valvar sinuses are then formed by ongoing proliferation of cells into the distal outflow tract from the second heart field. The leaflets are initially thickened structures filled with an abundant extracellular ground substance, densely populated with endocardial-derived mesenchymal cells, bordered by a cuboidal endothelium on the arterial surface, and by a flattened, streamlined endothelium on the ventricular surface [129,140]. After the sinuses are fully excavated, the thickened structures remodel into the thin fibrous tissue characterizing mature semilunar leaflets. Valve remodeling is a slow process that even at birth may be incomplete. The mechanisms that guide this remodeling are incompletely understood. The only neural crest–derived cells present in the developing semilunar leaflets are at the junctures of the zones of apposition between them and the arterial wall of the valvar sinuses, and then only in all components of the aortic root [141,142]. These locations may hint at links between neural crest abnormalities and abnormalities of valvar formation or location.

An animal model of both aortic and pulmonary valve aplasia is found in mice deficient in the *NFATC* gene [143,144]. NFAT proteins are transcription factors known to be important mediators of intracellular calcium signaling in the immune system, nervous system, myocardium, and skeletal muscle [145,146]. NFAT signaling is initiated by calcineurin, the primary intracellular target of cyclosporine and FK506. During heart development in mice, expression of the *NFATC* gene is limited to a subset of cells in the endocardium, but beyond this observation the mechanism of contribution of NFATc to arterial valvar development is unknown. Although rare, aplasia of both arterial valves has been described in humans [147,148]. Additionally, dysplasia of one or another arterial valve is one of the most common forms of congenital heart disease, either seen in isolation, as in the aortic valve with only two leaflets, or in conjunction with other defects such as tetralogy of Fallot, tetralogy of Fallot with absent pulmonary valve syndrome, or pulmonary atresia. Valvar problems are also encountered in a wide variety of left ventricular outflow tract defects that include aortic valvar hypoplasia and hypoplastic left heart syndrome as their most severe manifestation. Mutations in the Notch/Jagged pathway genes, as well as EGFR, have been implicated in bicuspid aortic valves, and as contributing factors to accelerated aortic valvar calcification [136,149]. Recent work targeting these pathogenic signaling pathways may serve as novel adjunctive therapies in the future [150]. The anatomy of the calcified aortic valve is unique such that the degree of calcification varies between the ventricular and arterial surfaces of the leaflets. Whether this reflects a difference in embryology, is affected by blood flow dynamics, or is a consequence of genetic background remains to be clearly elucidated. Indeed, evidence is mounting that it is multifactorial.

Aortopulmonary Septation

The aortopulmonary septum is initially formed as a protrusion from the dorsal wall of the aortic sac between the fourth and sixth aortic arches (Figure 1.11) [128,138]. The extracardiac origin of this mesenchyme has been well recognized since the late 1970s [151]. Kirby identified a population of neural crest cells that contribute to aortopulmonary septation [35]. This protrusion grows toward the opposing cushions that are forming within the persisting myocardial component of the outflow tract. As the cushions themselves initially fuse distally, so an embryonic aortopulmonary foramen can be recognized between the protrusion and the fused cushions. It is obliteration of the foramen that completes the formation of the nonmyocardial intrapericardial arterial trunks. Only after this process is complete is it possible to recognize the two prongs of mesenchymal condensations that penetrate caudally to occupy the proximal cushions, which are themselves unfused at this stage. Septation then moves proximally toward the heart and is accomplished by

fusion of the cushions containing the prongs of neural crest–derived mesenchyme. With ongoing maturation, the arterial trunks and roots achieve their components of neural crest–derived smooth muscle cells, so the components of the outflow tract separate one from the other. It is failure to close the embryonic aortopulmonary foramen that produces the various forms of aortopulmonary window. These are found only in the presence of normal separation of the arterial roots and ventricular outflow tracts. Failure of fusion of the outflow cushions themselves is the cause of common arterial trunk.

Development of the Aortic Arch

The great vessels are the conduits for blood to flow from the heart to the body and therefore must be formed and functional at the time of initiation of embryonic circulation, which takes place at approximately day 20–22 of gestation in humans. The vessels of the embryo are formed by a process called vasculogenesis. Vasculogenesis occurs by aggregation of pre-endothelial cells, or angioblasts, into networks of small endothelial channels. This contrasts with the process of building vessels by sprouting growth or branching, called angiogenesis. These endothelial channels assume arterial or venous identity based on distinct molecular signatures, even before the initiation of circulation. The dorsal aorta and aortic arches develop by fusion of independently formed regional arterial vasculogenic networks. Loss of arterial-specific markers adversely impacts the maturation of this nascent arterial network, and can result in embryonic lethality [152]. After communications between the arterial and venous networks are established, the definitive lumen is formed through merging of the small endothelial passages into larger channels [153]. The channels are functional vessels composed of only endothelial cells. Mesenchymal cells in the descending aorta and neural crest cells in the aortic arch region are then recruited to form the smooth muscle cells of the media of the developing arteries [154–156]. These enveloping events require signaling through extracellular proteins known as angiopoietins via the Tie1 (TIE) and Tie2 (TEK) receptors in endothelial cells [157]. The transcription factor KLF2 (LKLF) has also been shown to be necessary for formation of the tunica media in embryonic vessels [158].

Initially, the embryonic arterial circulation is bilaterally symmetric, and consists of multiple pairs of aortic arch vessels connecting the heart outflow to the paired dorsal aortas (Figure 1.11). The dorsal aortas are initially paired for the full length of the embryo. Fusion of the paired aortas into a single structure begins distally and progresses in retrograde fashion. As development proceeds, the paired first, second, third, fourth, and sixth aortic vessels, and the dorsal aortas, undergo an intricate series of transformations. The first and second aortic arch vessels regress, remaining patent only as capillary structures. The dorsal aorta between the third and fourth aortic arch vessels, known initially as the carotid duct, regresses completely, leaving no remnant, resulting in the paired third aortic arch vessels becoming the only source of blood flow from the intrapericardial aorta to the head of the embryo. The third aortic arch vessels become the precursors of the definitive common carotid arteries. The right dorsal aorta completely regresses at the site of dorsal aortic bifurcation. This leaves the right fourth aortic arch vessel to become a short stub connecting the right seventh intersegmental, the future subclavian artery, to the aortic sac. The left sixth aortic arch vessel becomes the arterial duct, with the right and left pulmonary arteries canalizing in the ventral pharyngeal mesenchyme, taking their origin from the floor of the aortic sac. The left dorsal aorta remains widely patent throughout its length, but remodels so that the definitive left fourth and sixth aortic arch vessels, along with the left seventh intersegmental artery, the future left subclavian artery, all connect to the left dorsal aorta [159]. Understanding these developmental concepts and relationships is helpful in planning repair of abnormalities of the great arteries as well as vascular rings. Despite the generally superb imaging now provided by echocardiography, computed tomography angiography, and magnetic resonance imaging that accompanies patients with aortic patterning defects, one is occasionally surprised by intraoperative findings. A basic knowledge of great vessel developmental derivatives can help troubleshoot an intraoperative surprise, and potentially limit the dissection required to reveal relevant operative anatomy.

The vertebral arteries are derived from anastomoses between the seven cervical intersegmental arteries. After continuity is established between the intersegmental arteries, their connections to the dorsal aorta regress, apart from the connection of the seventh intersegmental vessel, which becomes the subclavian artery. It is this process that accounts for the subclavian origin of the definitive vertebral arteries.

Neural crest cells are critical to the normal pattern of regression or maintenance of aortic arch vessel patency [3,32]. When neural crest cells are physically ablated in chick embryos, vascular patterning is abnormal in 100% of experiments, although the specific pattern of ablation-induced abnormalities is not predictable. Neural crest cells invade and replace the original tunica media of the aortic arch vessels, but it is not known by what specific mechanisms neural crest cells determine the future vascular pattern. Several genetic models of abnormal aortic vessel patterning that are not yet linked to neural crest abnormalities have been identified in mouse and zebra fish. Genetic engineering experiments

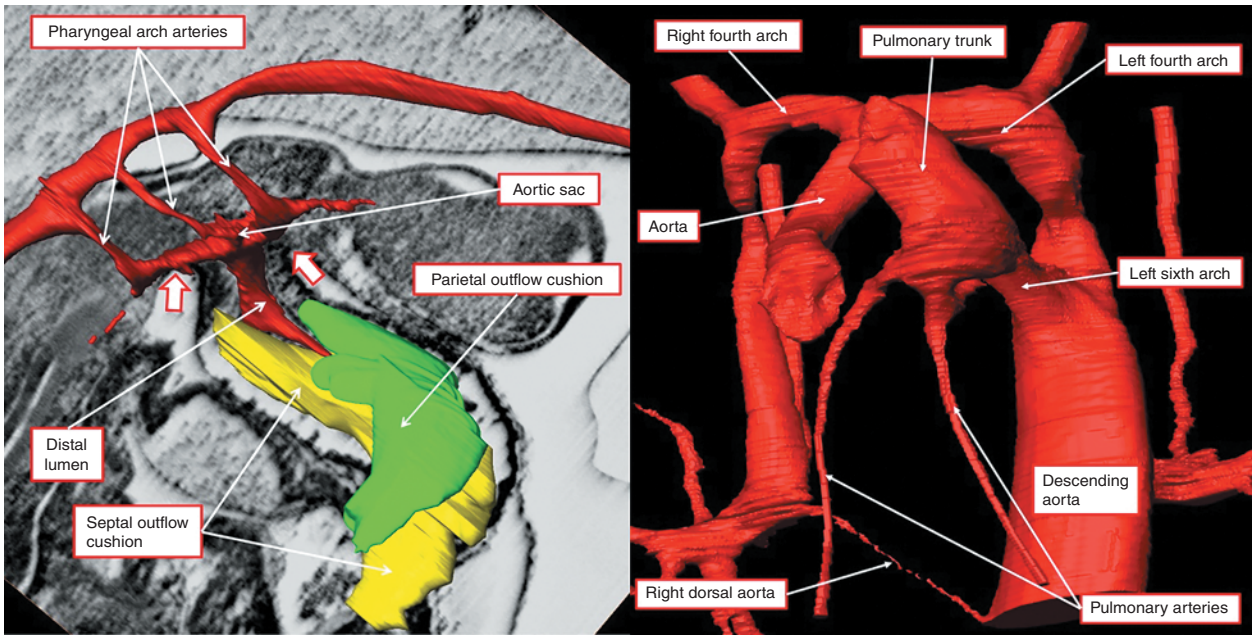

Figure 1.11 The images show how remodeling of the branches from the aortic sac produces the extrapericardial arterial channels. The left-hand panel is a reconstruction of a mouse embryo.

in mice involving the closely related transcription factors mesoderm Forkhead Box C1 (Foxc1) and mesenchyme Forkhead Box C2 (Foxc2) demonstrate cardiovascular phenotypes that include coarctation of the aorta, interrupted aortic arch, ventricular septal defects, and, in the case of Foxc1, thickening and partial fusion of arterial valvar leaflets [160]. It is also interesting to note that abnormalities with left fourth pharyngeal arch artery maturation are disproportionately represented in clinically observed arch artery anomalies, such as interrupted aortic arch, right-sided aortic arch, and cervical arch.

Coronary Arterial Development

As the epicardial layer of cells extends over the heart, an extensive acellular extracellular matrix layer appears between the epicardium and the myocardium. If migration from the proepicardial organ is physically disrupted in chick embryos, then no epicardium develops, and there is failure of formation of the subepicardial extracellular matrix [161]. More recent experiments using genetic lineage-tracing techniques in mice confirm that proepicardial-derived endothelial cells contribute significantly to the coronary vasculature [162,163]. The subepicardial space becomes populated by mesenchymal subepicardial cells, generally accepted to provide the precursors of cardiac fibroblasts, and coronary vascular smooth muscle cells. The subepicardial extracellular matrix accumulates to its greatest degree in the AV groove. In addition to being a key site for coronary vasculogenesis, the mesenchymal cells that come to populate the AV groove matrix will form most of the fibrous insulating tissues between the atria and the ventricles [100]. All the cell populations just described as invading the myocardium and/or subepicardial extracellular matrix have come to be known as epicardial-derived cells (EPDCs) [103]. As identified by lineage markers, EPDCs migrate into the matrix of the AV grooves, and subsequently into the AV endocardial cushions. In addition to entering the AV cushions, EPDCs also migrate into the myocardium and subendocardium. Specific possible morphogenetic roles of the myocardial and subendocardial EPDCs have not been determined, but abnormalities of the compact myocardium, AV cushions, and coronary vasculature have all been documented in their absence [164].

The subepicardial space is the site of origin of the vascular plexus of the coronary vessel precursors. The exact origin of the coronary endothelium is controversial. Reports have suggested that these cells may originate from the AV groove or the endothelium of the systemic venous sinus [165], from endocardium of the heart [166], or from circulating angioblasts. There are three sequential and overlapping phases of nutrient delivery to the myocardium during embryogenesis of the heart [117,159]. The first phase is associated with the development of an extensive network of intratrabecular spaces lined by endocardial cells, through which nutrient flow to the myocardium is hypothesized to occur. The second phase is the development of a subepicardial

plexus of endothelial-lined channels that penetrate the myocardium. A subset of these channels communicates with the intratrabecular spaces. The third stage is regression and coalescence of the vascular subepicardial network into muscular arterial channels. As soon as the vessels are readily identifiable, they are noted to penetrate the ventricular and atrial walls, where they establish a mid-myocardial network. The vessels spread to the ventral surface of the heart and follow the grooves, especially the AV grooves, to the outflow tract, where they form a plexus in the myocardial sheath surrounding the developing arterial roots. Coalescence of vessels, and capillary outgrowth from the developing aortic root, results in formation of the proximal coronary arteries [39,167,168]. Abnormalities of this process likely contribute to the pathogenesis of several surgically important disease phenotypes, including intramural coronary orifices, abnormalities of coronary positioning in both normally related and transposed great vessels, and anomalous origin of the coronary arteries from the pulmonary trunk.

Conduction

The sinus node is formed at the junction of the superior caval vein with the expanding right atrial appendage. The cells that will condense to become the node occupy the epicardial tissue of the terminal groove [169]. The AV node has a more complex developmental evolution, since it cannot achieve the structure seen postnatally until after the completion of atrial septation. Indeed, new imaging techniques suggest additional complexity [170]. The muscularization of the vestibular spine and mesenchymal cap forms the anteroinferior buttress that anchors the septum against the insulating tissues of the central fibrous body. The continuity between the compact component of the node and the ventricular parts of the atrioventricular conduction axis are present earlier. They are part of the ring of cardiomyocytes that surrounds the embryonic interventricular communication. Part of this ring becomes sequestrated within the vestibule of the right atrioventricular junction, providing the potential for alternative nodes to be formed around the orifice of the right AV valve [171]. In the normal heart, it is the AV conduction axis that remains as the solitary muscular connection between the atrial and ventricular masses [115,172]. Invagination of the mesenchyme of the atrioventricular groove serves to encase the future bundle of His in the insulating tissue of the central fibrous body. Differentiation of ventricular components of the conducting tissues occurs by recruitment of "working" myocardium into the conduction lineage [173,174]. It is the trabeculations of the inner layer of the initial ventricular walls that are used to connect the proximal bundle branches, derived from the primary ring, with the ventricular free walls [175]. Subsets of these trabeculations remain as elements of the conduction tissue in the mature heart [176].

Conclusion

Intricate spatial and temporal regulatory mechanisms govern morphogenesis of the heart. The efficiency of these regulatory mechanisms is evident when one considers that, despite the complexities involved, abnormal development of the heart is seen in only a small proportion of normal pregnancies. A greater appreciation of the developmental mechanisms can enhance our understanding of congenital heart diseases. As physicians, such knowledge can help us to care for these complex patients.

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