2

Cardiac morphogenesis

Margaret L Kirby and Karen Waldo

Introduction

Cardiogenic mesenchyme is established at gastrulation in two heart fields lying bilaterally at the anterolateral region of lateral plate mesoderm.[1] In response to appropriate inductive signals from the endoderm, cells in the splanchnic layer of mesoderm in this region begin to express the transcription factor Nkx2.5 and subsequently differentiate into myocardial cells.[2,3] Endocardial cells are also thought to arise from the cardiogenic mesenchyme, but cells from other regions of the mesoderm also contribute.[4,5] The myocardial and endocardial cells arrange themselves into the concentric epithelial layers of a bilayered tube at the embryonic midline. An additional acellular layer called the cardiac jelly separates the cellular layers. This is partially produced by the myocardium in response to endocardial signaling, with some components such as fibronectin added by the endocardium.[6] The cardiac jelly

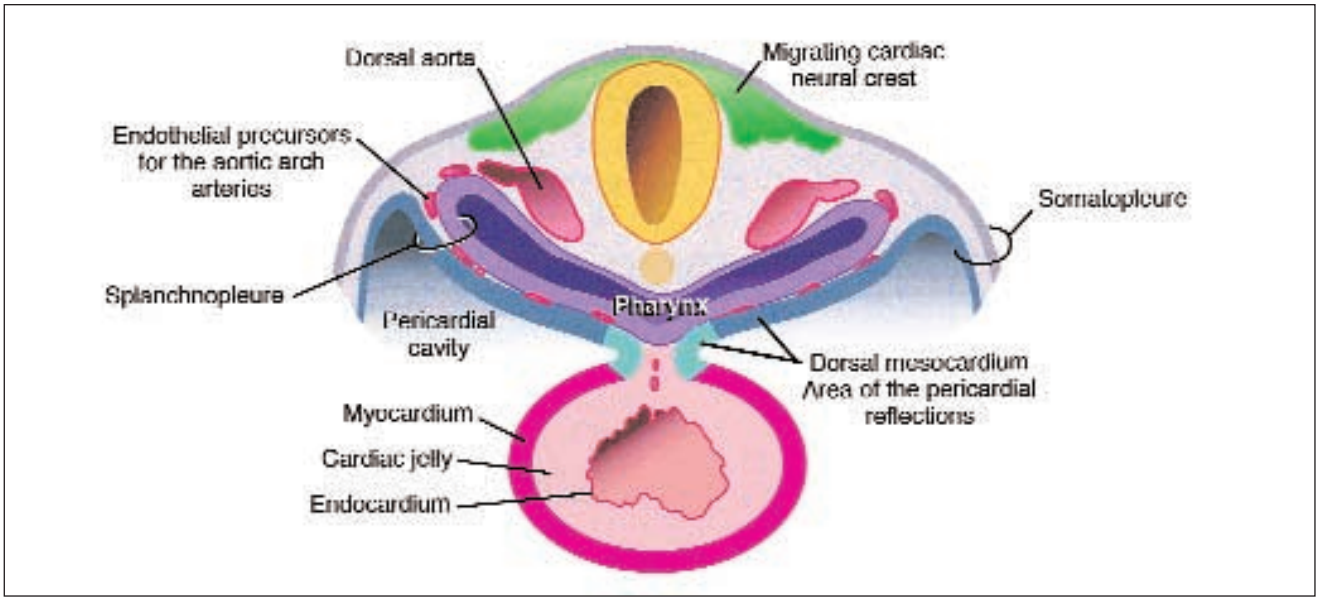

Figure 2.1
In the early embryo, a single heart tube develops in the ventral midline from the fusion of bilateral heart tubes. The heart tube is suspended from the pharynx into the pericardial cavity by a dorsal mesocardium that forms from a reflection of the pericardium from the roof of the pericardial cavity (see also Figure 2.2). The dorsal mesocardium is continuous dorsally with the pericardium and ventrally with the myocardium of the heart tube. Between its two leaves, splanchnic mesenchyme contains endocardial precursors, which will form the aortic sac that connects the heart with the arch arteries. The heart tube consists of three layers: the outer myocardium; the inner endocardium surrounding the cardiac lumen; and the cardiac jelly that intervenes between the inner and outer layers. Dorsal to the newly formed pharynx, bilateral dorsal aortae have formed. Within the splanchnopleuric mesenchyme, vascular endothelial precursors will coalesce adjacent to the pharyngeal endoderm to form the arch arteries that will connect the dorsal aorta to the aortic sac. As the neural tube closes, cardiac neural crest cells begin their migration toward the pharynx and heart.

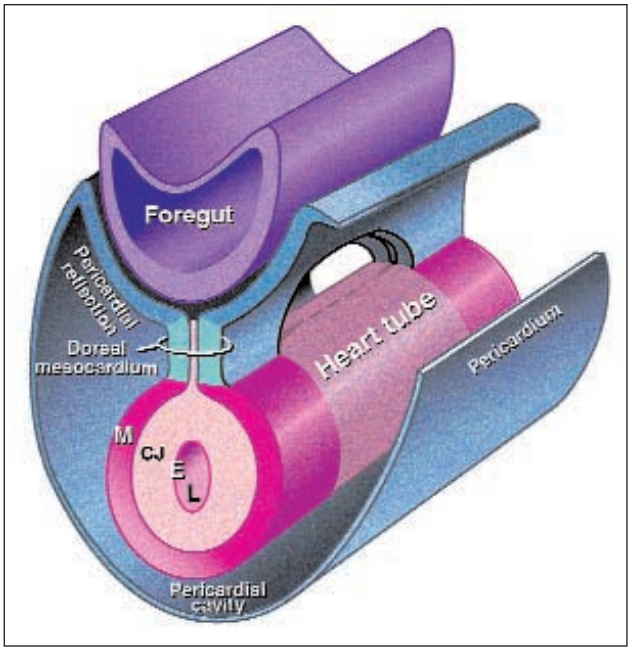

Figure 2.2
As the heart tube lengthens, the mesocardium loses its attachment to the original heart tube except at the inflow and outflow poles. The myocardial layer closes dorsally as the mesocardium loses its connection to the heart. The heart tube lengthens at both ends by accretion of tissue where the dorsal mesocardium is still attached to the heart. M, myocardium; CJ, cardiac jelly; L, lumen; E, endocardium.

within the heart tube is devoid of inclusions, whereas the comparable extracellular material extending into the pharynx and underlying the pharyngeal endoderm is rich in high-density amorphous material and fibrils.[7] All together these three layers, the myocardium, endocardium and cardiac jelly, form the initial heart tube (Figure 2.1), which provides the foundation for development of the four-chambered heart. The inflow and outflow portions of the tubular heart are added from the extremities of the initial heart tube, where the myocardium is continuous bilaterally with the splanchnic mesoderm of the pericardial reflections (Figure 2.2).

Only the components of this basic heart tube are formed from the cardiogenic mesenchyme. To complete heart development, at least two extracardiac sources provide cells for the developing heart. These include the epicardial organ,[8] a bud from the liver mesenchyme; and neural crest and Ventrally Emerging Neural Tube (VENT) cells originating from the nervous system.[9,10]

Prior to gastrulation, the cells that ultimately give rise to the myocardium and endocardium of the primitive heart tube are located bilaterally in the epiblast, midway along the length of the presumptive primitive streak (Figure 2.3).[1,11,12] The cells are induced to form the heart field during gastrulation by signals from the node.[12] After passing through the primitive streak, these cells migrate craniolaterally as lateral plate mesoderm.[11] Cardiogenesis requires another induction by factors produced by the anterior endoderm, which is in close proximity to the anterolateral lateral plate mesoderm.[13–16] A number of signaling molecules have been proposed to induce cardiogenesis; the most rigorously tested are bone morpho-

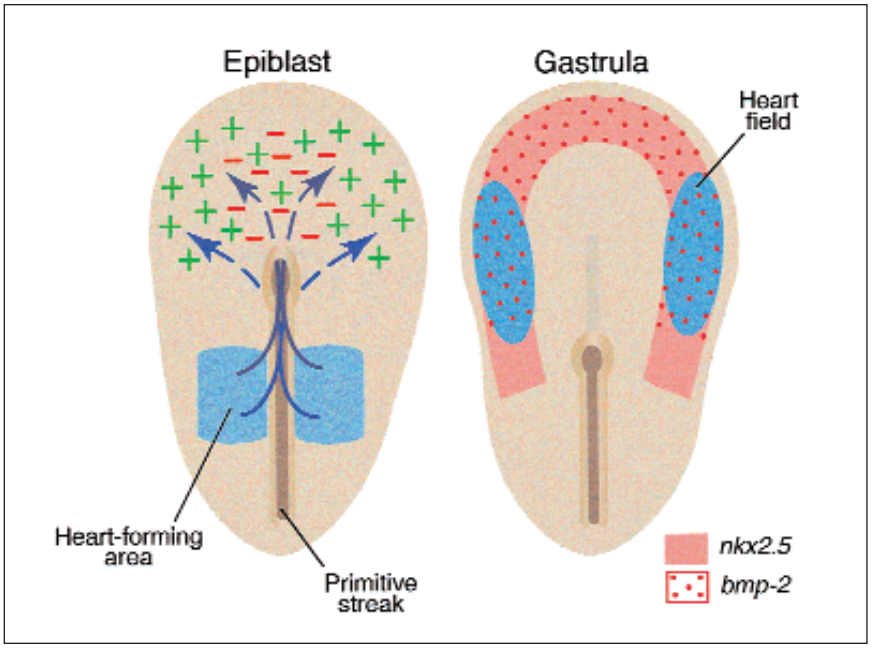

Figure 2.3
In the epiblast, precursors of the myocardium and the endocardium lie on either side of the primitive streak. These cells egress through the primitive streak and migrate to form the anterior lateral mesoderm where they encounter positive and negative signals from surrounding tissues.[13] As gastrulation proceeds, the myocardial phenotype is induced by signals from the endoderm such as BMP-2 and FGF.[17,106]

genetic protein (BMP) and fibroblast growth factor (FGF) family members. In combination, these proteins can mimic the cardiogenic inductive activity of anterior endoderm.[17] The entire heart field has the potential to contribute to the heart tube. However, inhibitory signals that reach the medial portions of the heart field from the neural tube respecify the most medial and anterior cells to form the dorsal mesocardium and a portion of the pericardium.[2,18,19] Thus, induction of heart from the mesoderm requires both positive and negative signaling—first, to induce heart formation; and second, to restrict the size of the heart progenitor pool.

One of the earliest genes expressed is Mesp1 which encodes a basic helix-loop-helix (HLH) protein expressed in all mesoderm during early gastrulation. Mesp1-expressing cells migrate from the primitive streak and are incorporated into the head mesenchyme and heart field. Disruption of Mesp1 expression leads to cardia bifida, highlighting the importance of a sound mesodermal basis for heart development.[20] As the pericardial coelom forms in the lateral plate mesoderm, N-cadherin is restricted to the central areas of mesoderm, defining the splanchnic (cardiogenic) from the somatic mesoderm, to form the pericardial coelom. Premyocardial cells begin to express N-cadherin, a transmembrane receptor that binds components in the extracellular matrix, on their apical surfaces that abut the pericardial coelom.[21] N-cadherin continues to be expressed in the myocardium throughout early development of the tubular heart, but it is absent from the cells close to the endoderm that will form endocardium.[22] The N-cadherin localized to apical surfaces is later incorporated into adherens junctions of contractile tubular heart. Premyocardial cells at the onset of differentiation also express N-CAM, a transmembrane protein that mediates cell–cell cohesion.

Myocardium

Genes expressed by the early myocardium

Members of the GATA and Nkx2 families have been shown to be of particular importance for early specification of the myocardium and for regulating expression of genes that encode the proteins of the contractile apparatus. The promoters of the genes encoding proteins that subserve excitation–contraction coupling are mostly not known, but presumably these promoters will contain binding sites for Nkx and GATA family transcription factors. The Nkx2.5 gene, considered to be the earliest marker of cardiac specification, is first expressed in the lateral plate mesenchyme almost as soon as the newly gastrulated cells are near the anterior endoderm.[13] Induction of cardiogenesis is accompanied by a decrease in expression of p-globin, suggesting that myocytes and blood-forming tissues have a common progenitor. By the time myocardium and endocardium form distinct layers, Nkx2.5 expression is confined to the myocardium.[13]

GATA 4, 5 and 6 genes code for zinc finger transcription factors. These GATAs are expressed in the cardiogenic mesenchyme, overlapping with, but in a wider area than, Nkx expression. All three GATAs are expressed in the myocardium and endocardium of the developing heart, but it is not clear whether they control differentiation of the cellular components of the heart. When the GATAs are deficient, major patterning defects, e.g. cardia bifida, occur in formation of the cardiac tube.[23] GATA 5 follows the same pattern as the other GATAs. It becomes restricted to the atrial endocardium, but is not expressed in the heart during late fetal and postnatal development.[24] In vitro, GATA 5 binds to the functionally important CEF-1 nuclear protein binding site in the cardiac-specific slow/cardiac troponin C (cTnC) transcriptional enhancer, and it has been shown that overexpression of GATA 5 transactivates the cTnC enhancer in noncardiac muscle cell lines.[24] However, the presence of transcriptional activity in vitro should be interpreted cautiously until similar activity can be shown in vivo.

Myofibrillogenesis and trabeculation

Local and regional variations and right side dominance in myofibrillar patterns can be seen during looping. Myofibrils near the lumen are primarily circumferential bands, while those near the pericardial surface are longitudinal bands. There appear to be regional variations in myofibrillar organization in areas associated with cardiac cushions, trabeculae and chamber walls. Myofibrils in the ventricle and outflow tract are more mature than those in the atrial wall.[25]

Fetal and adult hearts rely mostly on the compact myocardial layer; however, in the embryonic heart, trabeculae play a crucial role in contractile function. The myocardium of the early heart tube initially provides a smooth inner surface. During looping, outer compact and inner trabeculated layers can be distinguished. As development proceeds, abundant trabeculae appear throughout both ventricular cavities, and the trabecular patterns and intertrabecular spaces become ventricle-specific.[26] The trabeculae begin in a radial arrangement, but later become spirally oriented and this persists in a simplified form into adulthood.[26]

The formation of trabeculae depends on a heuregulin/neuregulin signal from the endocardium. The myocardium expresses ErbB2 and 4 proteins that are receptors for heuregulin.[27,28] In mouse embryos that do

not express either the heuregulin signal or the ErbB receptors, trabeculae do not develop and the embryos die early in development.

Conduction system

The working myocardium is thought of primarily as the contractile tissue of the heart, but it also plays the central role in generating the conduction system, which allows the heart to maintain a coordinated, rhythmic wave of contraction.[29] Both the central (nodes) and peripheral conduction tissue (His bundle, bundle branches and Purkinje cells) are recruited from the cardiomyocyte pool with no contribution evident from any extracardiac source.

The Purkinje cells that form the conduction system are recruited from the working myocardium by paracrine cues emanating from the coronary vasculature.[30] Both working myocardium and Purkinje cells can share progenitors. The progenitors of the nodal tissue are thought to be set aside from the cardiomyocyte pool earlier, so that nodal cells and working myocardial cells do not share progenitors.[31] In vitro experiments have shown that vascular endothelin can convert embryonic myocytes to Purkinje cells. Furthermore, inhibition of coronary arterial development results in a significant reduction in the density of intramural coronary arteries and the Purkinje fibers that are associated with these arteries.[32] Finally, the activation of coronary arterial branching induced by retroviral expression of FGF-2 results in the development of ectopic Purkinje fibers adjacent to the induced coronary arteries.

The early conduction system is acetylcholinesterase-positive and expresses different myosin isoforms, allowing visualization from early stages of development.[33,34] At later stages, PSA-NCAM and HNK-1 cell surface glycoproteins involved in cell–cell and cell–substrate interactions are expressed throughout the conduction tissue.[35,36] NT3, a neuropeptide, colocalizes with these markers, suggesting that these specialized myocardial cells adopt aspects of a neural phenotype.[37] Because both neural and conduction cells are specialized to conduct electrical impulses, this may be a good example of convergent evolution.

Endocardium

Genes expressed by the early endocardium

The endocardium of the primitive heart tube is derived, at least in part, from the precardiac lateral plate mesoderm and is, like the myocardium, induced by signals produced by the anterior endoderm.[13] This has been confirmed by experiments in vitro in which anterior endoderm induced the expression of the endocardial markers cytoactin, fibrillin 2 and QH-1 in cultured precardiac mesoderm.[38] Expression of the endocardial markers is first observed in the cardiogenic mesoderm during late gastrulation, around the stage at which myocardial markers are initially expressed. A second population of endocardial cells originates outside the putative heart field and merges with those found in the precardiac mesoderm to form the vascular plexus, which soon thereafter becomes the endocardial epithelium of the primitive heart tube.[39] Finally, lineage studies have shown that the endocardium of the conus and truncus is derived from the lateral plate mesoderm distinct from the cardiogenic plate.[5]

Much less is known about the expression of early endocardial genes. Notch is expressed in the early endocardium.[40] Notch is a transmembrane receptor that is expressed in segregating tissues,[41] so it is intriguing that it is expressed in the endocardium, as it segregates itself from the myocardium. Some genes are expressed in all of the cardiogenic mesenchyme and later become localized to either the myocardium or the endocardium. GATA 5, which is expressed in cardiogenic mesenchyme containing both pre-myocardium and pre-endocardium, is later localized to the endocardium.[42]

In addition, many pan-endothelial markers are expressed by the endocardium. For example, flk-1, a tyrosine kinase receptor for vascular endothelial growth factor (VEGF), is expressed in the precardiac mesoderm.[43–46] Both flk-1 and VEGF null mutant mice fail to form a proper endocardial epithelium.[43,44,47,48]

Hex expression marks the anterior end of the embryo and is the earliest marker of the head.[49] It is expressed in the pharyngeal endoderm and later in the endocardium. Hex transcripts are also detected within blood islands and vascular endothelial cells as vessels form, suggesting a common lineage of hematopoietic cell lines with the endothelial/endocardial lineages.[50]

PECAM-1 is a cell surface glycoprotein that mediates endothelial cell–cell adhesion, and presumably performs the same function in the endocardium.[51] α_4-β_4-integrin mediates tight adhesion of endocardial cells to basement membranes.[52]

After it is formed, the endocardium contributes cells to the mesenchyme of the endocardial cushions. One of the factors important in this induction is transforming growth factor-β (TGFβ). Endoglin is a TGFβ receptor which can be detected in early endocardium, but it is not expressed by the myocardium. It reaches very high levels on the endocardial cushion tissue mesenchyme, and can be seen during heart septation and valve formation, after which it decreases as the valves mature.[53,54]

Teratogenic studies and mutations in the retinoic acid receptors in mice have shown that the heart is dependent on critical levels of retinoic acid for normal development. Interestingly, P450RA is a retinoic acid-degrading enzyme with expression restricted to the endocardium.[55]

Endocardial cushion formation

The initially cell-free cardiac jelly becomes populated with cells to form endocardial cushions. This mesenchyme participates in septation of the outflow tract and atrioventricular canal, and is an important source of cells for valve development.[56]

Some but not all endocardial cells undergo an epithelial–mesenchymal transformation to generate the cells that populate the cardiac jelly.[57] The epithelial–mesenchymal transformation does not occur throughout the heart tube, owing to the lack of an inductive signal from the myocardium. The signal is generated only in the atrioventricular canal and outflow tract and so cushion mesenchyme does not form in the atrial and ventricular regions.[58,59] The entire atrioventricular and a portion of the outflow endocardial cushions are formed when epithelial endocardial cells delaminate and take up residence in the cardiac jelly as mesenchyme.[60] The initiation and completion of this process depends on at least two signaling pathways. In the first step, the myocardium releases a factor that induces an alteration in gene expression in some endocardial cells. Because ErbB3, the receptor for heregulin, is needed for formation of the cushions, this may be the initial signaling pathway.[61] Other candidates for initiating the process include BMP-2[62] and ES130, an EDTA-soluble factor that is found in 30-nm particles in the region of the cardiac jelly where these epithelial–mesenchymal transformations occur.[63] Antibodies against ES130 effectively block the inductive effect of myocardium on endocardial cell transformation. TGF β_2 and TGF β_3 are involved in the process of epithelial-mesenchymal transformation. TGFβ_2 appears to mediate the initial endothelial cell separation, while TGFβ_3 causes the changes in cell morphology that accompany migration.[64] Madh6 is one of the intracellular signaling pathways for TGFβ, and mice mutant for Madh6 have multiple cardiovascular abnormalities including hyperplastic cardiac valves.[65]

In the atrioventricular canal the cushions are formed entirely from the endocardium. However, the outflow cushions are only partially formed by the endocardium. The nonendocardially derived mesenchyme of the outflow cushions comes from at least two other sources that include neural crest-derived mesenchyme and splanchnic mesenchyme.[9,66]

Extracardiac contributions to heart development

Almost all of the early work on heart development was done with the idea that the cardiogenic mesenchyme provided all of the progenitor cells needed for development of a mature four-chambered heart. Work over the past 20 years has shown definitively that several extracardiac sources of cells are necessary for normal heart development. It is probably too early to say definitively that all of the extracardiac tissues that contribute to heart development are known. The extracardiac sources that are now recognized include two populations from the neural tube (neural crest cells from the dorsal neural tube and VENT cells from the ventral neural tube),[9,10] the epicardial organ (derived from liver mesenchyme[8,67,68]) and the spina vestibuli.[69]

Epicardial organ

The epicardial organ gives rise to the epicardium, which in turn generates the endothelial and smooth muscle cells that form the coronary vasculature, subepicardial mesenchyme and parts of the fibrous skeleton of the heart.[68,70–72] The epicardial organ arises as a bud near the venous pole of the tubular heart, and gradually envelops the entire heart (Figure 2.4). The outflow tract is the last to be covered. Cells from the epicardium undergo epithelial-to-mesenchymal transformation to generate a subepicardial mesenchyme.[70] A coronary vasculature network forms in the subepicardial extracellular matrix and envelops the entire heart. Cells derived from the epicardial organ are recruited into three distinct coronary vessel-associated cell populations: coronary smooth muscle, perivascular connective tissue and endothelial cells.[71] Capillaries near the developing aorta penetrate the aortic wall above the nascent semilunar valve. These capillaries enlarge to form the roots of the coronary arteries.[73,74] Smooth muscle precursor cells ensheath the coronary vessels and are induced to express a smooth muscle phenotype by neural derivatives of the neural crest.[75] Cardiac neural crest-derived parasympathetic ganglia seem to be essential to the survival of the definitive coronary vessels.[76]

Although the mechanisms that underlie mesenchymal recruitment to the coronary vasculature have not been explicitly determined, growth factors that are produced by the endothelial cells in the nascent capillaries, which include epidermal growth factor and platelet-derived growth factor-BB, may serve as inductive signals for this process.[77] A number of growth factors have been shown to be capable of inducing epithelial–mesenchymal transformation of the embryonic cells in vitro. FGF-2, EGF and

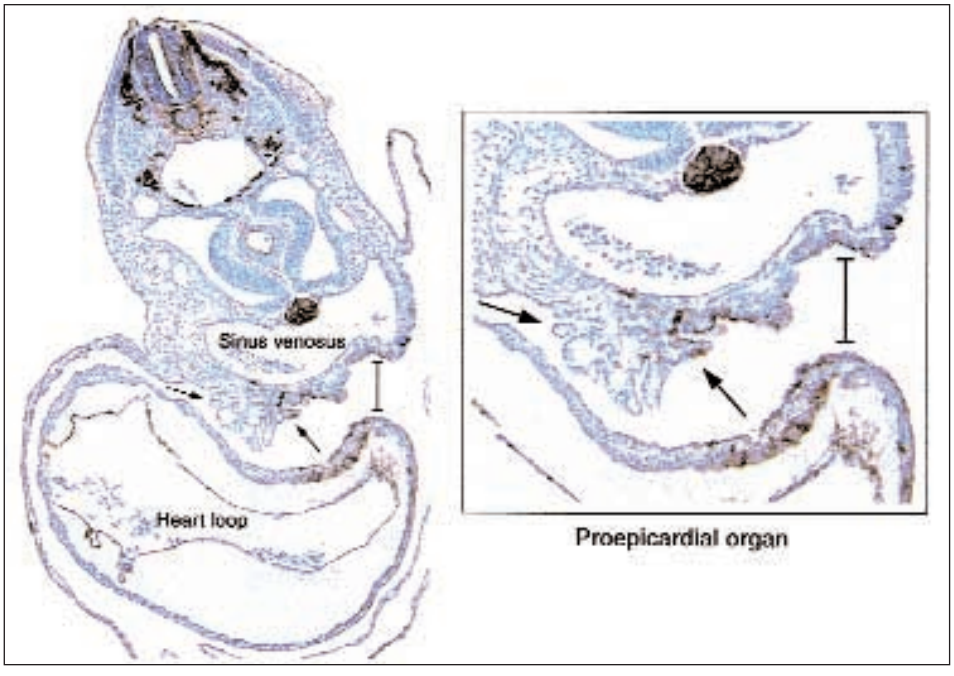

Figure 2.4
At stage 16 in the chick embryo, the proepicardial organ first appears as evaginating fingers that extend from the ventral part of the sinus venosus to the inner curvature of the heart loop. Epicardial precursors migrate from the tips of the fingers onto the surface of the myocardium to form an epithelial mantle. The heart becomes completely covered with epicardium by stage 26 and the epicardium and pericardium become continuous with each other at the pericardial reflection.

VEGF can each induce epicardial cells to invade collagen gels, a strong indicator of a mesenchymal phenotype.[71] Each of these growth factors is produced by the myocardium during this transformation. Given the spatial relationship of the myocardium to the epicardium, the myocardium may be the source of the signals that are required to stimulate the epicardial epithelial–mesenchymal transformation.

Cardiac neural crest

The cardiac neural crest originates from the dorsal neural tube. It contributes mesenchymal cells to the outflow tract septum, neuronal cells to the cardiac ganglia and smooth muscle cells to the great arteries.[9,78–80] Cells derived from the cardiac neural crest are also essential for correct remodeling of cardiovascular tissues and maturation of the excitation–contraction coupling apparatus of the myocardium.[81]

Ablation of the premigratory cardiac neural crest in chick embryos results in a set of defects collectively referred to as the cardiac neural crest ablation phenotype.[82] These defects include persistent truncus arteriosus, mispatterning of the great arteries and hypoplasia of the pharyngeal glands (thymus, parathyroid and thyroid). The morphological anomalies are accompanied by ventricular function deficiencies that include reduced ejection fraction caused by decreased calcium current and abnormal excitation–contraction coupling.[83–86] Altered hemodynamic properties in the aortic arch arteries, which also develop abnormally, were initially suspected to underlie these functional deficits. Other myocardial alterations that are observed in the same developmental window as the functional deficits include abnormal production of cardiac jelly, increased proliferation and disorganized myofibrils.[86] These characteristics of primary myocardial dysfunction occur prior to the stage at which the neural crest cells would have entered the heart in intact embryos. Thus, the influence of the neural crest on myocardial development is temporally and spatially isolated from its role in outflow tract septation and occurs while the neural crest is in the caudal pharyngeal arches.

The hemodynamic properties of the aortic arch arteries and the peripheral circulation are unaffected by cardiac neural crest ablation. Sophisticated methods that measure pressure, pressure gradients, impedance, vascular resistance, wall stresses and flow have failed to detect any deficiencies in these parameters in the aortic arch arteries of cardiac neural crest-ablated embryos (Farrell et al, unpublished results). These measurements tend to exclude the possibility that increased load causes depressed ventricular function in these embryos. While hemodynamic abnormalities cannot be completely excluded as a cause of myocardial dysfunction, other factors must play a more prominent role in generating the functional changes that have been observed in cardiac neural crest-ablated embryos.

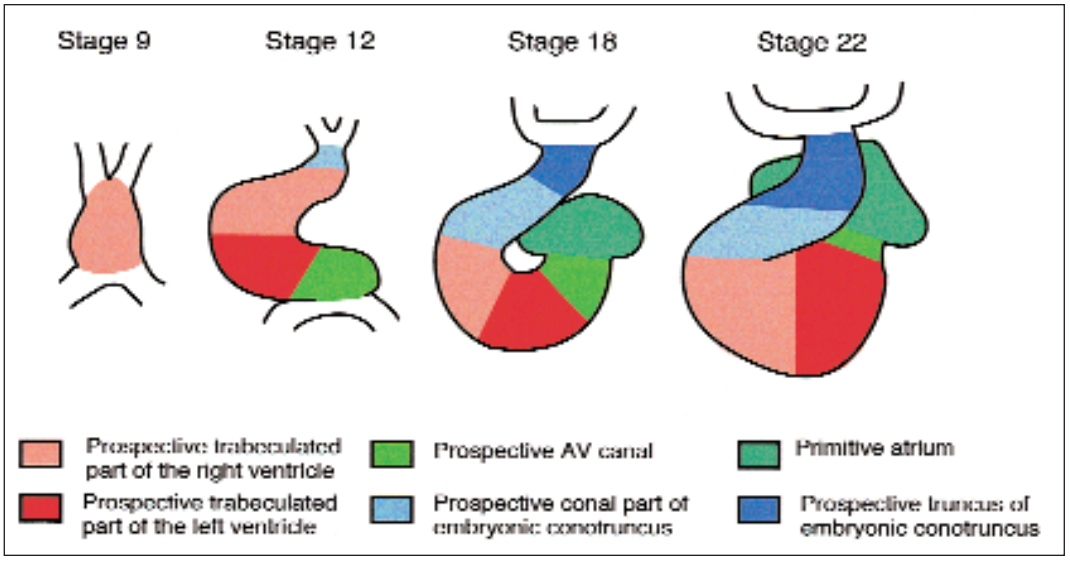

Figure 2.5
As looping occurs, the heart tube lengthens. When the heart tube first appears, only the presumptive trabeculated part of the right ventricle is present. The part that will become the trabeculated part of the left ventricle is added by stage 10. By stages 12–14, when the heart has bent to the right, a part of the conus and the future atrioventricular canal (AV canal) has appeared at opposite poles. The truncus and the atrium and sinus venosus are added by stages 18–22. By the end of the looping period the heart has completed the lengthening process and undergoes expansion and septation of the segments of the heart.

Organogenesis

Tubular heart

The tubular heart as it is initially formed is composed of three layers: myocardium, cardiac jelly and endocardium. If no further growth occurs, this tube will give rise only to the trabeculated portions of the right and left ventricles. There is dynamic growth or accretion of tissue at the ends of the tube to form the definitive inflow and outflow, i.e. atria, atrioventricular canals, and proximal (conus) and distal (truncus) outflow (Figure 2.5).[87] Epicardium begins to invest the looped tube during the later stages of incorporation of the definitive inflow and outflow.[8,67] The myocardial layer is 2–3 cells thick and begins contracting soon after the tube is formed. This constitutes the "working" myocardium and, as it proliferates to form compact and trabecular layers, some cells will be set aside from the working myocardium and become specialized for conduction myocardium.

During the period of lengthening by accretion of tissue, the tube undergoes looping. The loop creates a descending or inflow limb and ascending or outflow limb with a flexure or "inner curvature" between. The extremities of the tube converge so that the atrial and outflow portions are at similar craniocaudal levels. The atrial portion comes to rest posterior to the outflow portion of the tube. After chamber formation, septation is completed as the outflow wedges between the atrioventricular valves (reviewed by Kirby and Waldo[82]).

Chamber specification

Marking experiments have clearly shown that only the trabeculated portion of the future right ventricle is derived completely from the straight heart tube. The left ventricle and the inflow tract are generated from the caudal portion of the tubular heart while the outflow tract (conus and truncus) is generated from the region of the junction of the tubular heart with the aortic sac.[87,88] It is not clear what cell populations generate these additional segments of the heart, but it is known that the new segments are added during and after the establishment of circulation. In the chick, the heart tube is present at stage 9, the atrioventricular canal is incorporated into the tubular heart at stage 13/14 and the conus and truncus are added between stages 12 and 22.[87]

Although the mechanisms that underlie specification of the chambers remain largely unknown, atrial and ventricular identities are established early in development through expression of chamber-specific isoforms of the contractile proteins. Anterior–posterior polarity and the atrioventricular (AV) boundary can be seen early in development by differential expression of myosin isoforms, suggesting that the myocardial cells may already be diversified when they differentiate.[89] The AV boundary can be shifted by retinoids, indicating that the diversification remains plastic.[89] Blockade of retinoic acid synthesis with disulfiram produced hearts lacking the atrial chamber, and atrium-specific gene expression is controlled by retinoic acid so that retinoic acid must be excluded from ventricular precursors for correct specification of the ventricles.[90]

Typically, genes that are expressed in a chamber-specific manner are initially expressed throughout the heart tube and later become restricted to a particular chamber. This suggests that repression of gene expression is an important mechanism for regulating chamber specification. Irx4, a homeobox gene expressed in a ventricle-specific manner throughout development, may play a role in chamber identity. The Irx4 protein product activates the expression of the ventricular myosin heavy chain-1 while suppressing the expression of atrial myosin heavy chain-1 in the ventricles.[91,92]

Nkx2.5, initially activated throughout the myocardium, continues to be expressed during development. Interestingly, a distal cardiac enhancer contains a high-affinity binding site for GATA 4. While the enhancer is active throughout the myocardium through the looping stage, as the chambers are specified the enhancer is active only in the right ventricle, suggesting that maintenance of expression of a gene throughout the myocardium requires multiple regulatory regions that may be separable.[93]

Very few markers found distinguish the presumptive right from left ventricle. However, in the mouse two basic HLH transcription factors are expressed in a complementary manner. dHAND is expressed in the presumptive right ventricle and eHAND is expressed in the presumptive left ventricle.[94] Null expression of dHAND leads to absent development of the right ventricle and outflow tract.[94] Versican, a chondroitin sulfate proteoglycan, may also be involved in specification of the ventricular chambers. It is highly expressed in the trabeculated ventricular myocardium, while little is present in the compact zone of the myocardium or in the atrial myocardium.[95] In a mouse with a transgenic insertional mutation in the versican gene locus, the right ventricle and outflow tract do not form.[58] That this may be an error in chamber specification is also suggested by the absence of endocardial cushions, which depend on regional identity for delamination of the endocardial cells.

Septation

Four separate but related septation events divide the heart into four chambers with two outflow vessels. Prior to septation the inner curvature of the looped tube remodels to allow expansion of the atrioventricular canal to the right. This is important to establish right-sided flow of blood before septation. Concurrently the sinus venosus is shifted toward the right and subsequently is incorporated into the right atrial chamber.[96]

Superior and inferior atrioventricular endocardial cushions fuse to form right and left atrioventricular canals. Very little is known about the molecular biology of this fusion. In humans, defects in collagen type II are associated with a persisting AV canal.[97] The AV canal is most frequently associated with trisomy 21,[98] but the identity of the gene responsible for the cardiovascular phenotype in this syndrome is still not known.

The primary atrial septum grows from the back wall of the common atrial chamber to separate the right from the left atrium. A dense core of mesenchyme at the base of this septum, designated the "spina vestibuli," originates outside the heart in the somatic mesoderm of the body wall and extends through the dorsal mesocardium into the atrium.[69] The leading edge of the primary atrial septum grows toward the AV endocardial cushions and fuses with them, closing the primary ostium or foramen. Concurrently, as the primary ostium is closed a secondary ostium develops in the dorsocranial portion of the primary septum. The cranial atrial wall folds inward to form the secondary atrial septum, which does not fuse with the AV cushions leaving the fossa ovalis.[69] Defective development of the primary atrial septum associated with hand malformations has been described as Holt–Oram syndrome and has been attributed to mutations in Tbx-5, a T-box containing transcription factor.[99,100]

After looping is completed and trabeculations appear in the apical region of the common ventricle, the ventricular septum grows from the myocardium at the outer curvature of the heart loop.[101,102] The superior and inferior cushions of the AV canal are continuous with the primitive interventricular septum.[102] The definitive interventricular septum has been shown to be composed of the inferior AV cushion, which forms the basal portion of the septum in the region of the inlet; the superior endocardial AV cushion forms the basal portion of the septum in the region of the outlet; and the primitive interventricular septum forms the middle third of the basal septum and the entire apical region.[102] Final closure of the ventricular septum depends on the septation of the outflow tract.

Septation of the outflow tract occurs via three different mechanisms (Figure 2.6).[79,103] Several structures participate in outflow septation.[66] The endocardium of the distal outflow tract (truncus) continues as the endothelium of the aortic sac. The cephalic margin of the myocardial

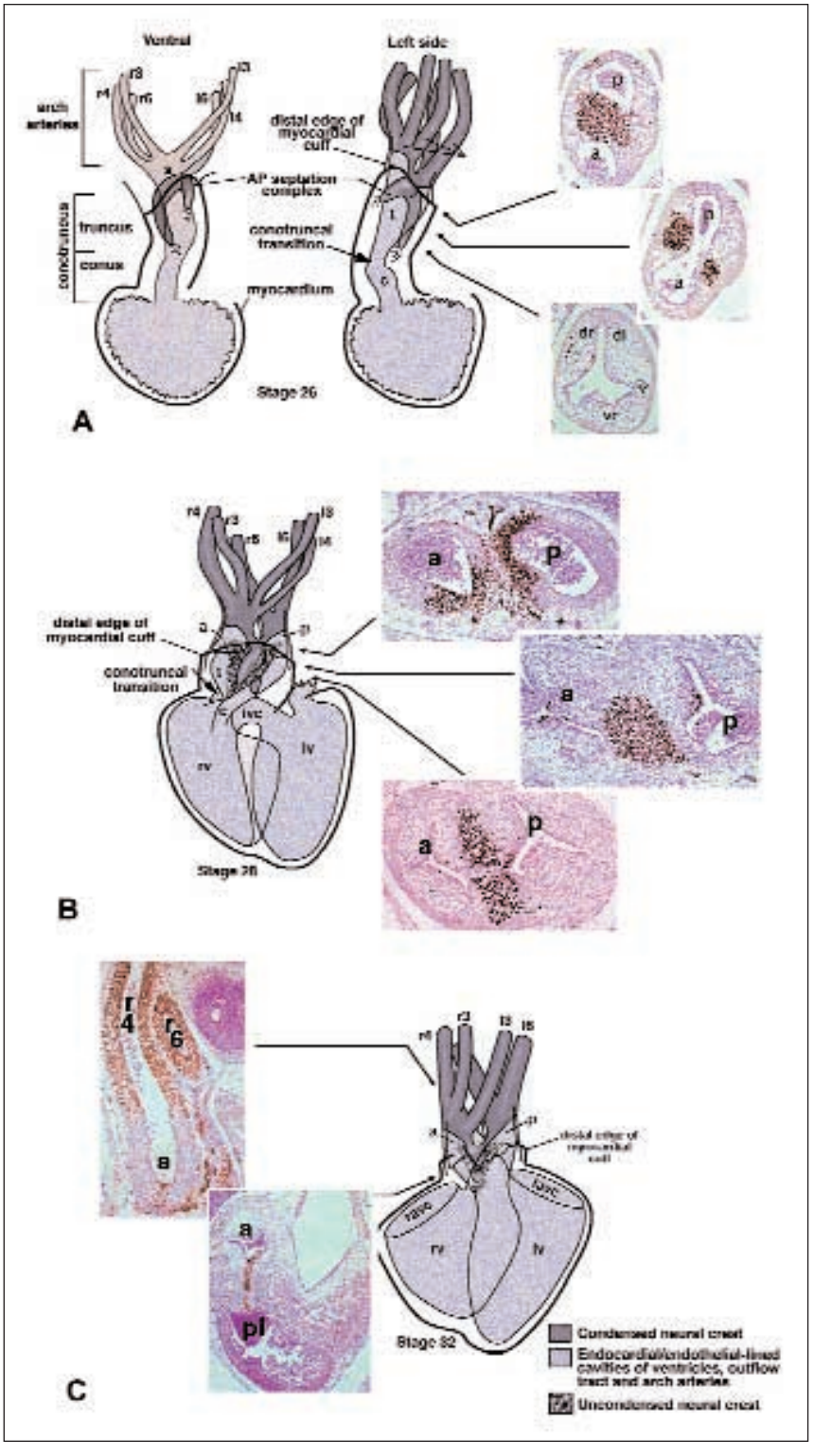

Figure 2.6
A summary of cardiac outflow septation in the chick embryo (from reference 79). Illustrations of the septation process have been combined with photomicrographs of histological sections from the cardiac outflow tract of quail-to-chick chimeras sectioned at the levels and in the planes indicated by the elongated leader lines with arrowheads. Quail cardiac neural crest cells are dark brown in the histology sections. (A) The aortic sac is septated by the bridging part of the aorticopulmonary septation complex between the origins of the fourth and sixth arch arteries. This process divides the aortic sac into the nascent aorta and pulmonary trunk. The condensed cardiac neural crest-derived mesenchyme of the aorticopulmonary septum forms an upside-down U that is continuous with the neural crest-derived mesenchyme forming the walls of the arch arteries. (B) The distal part of the conotruncus (truncus) is divided by the bridging part of the aorticopulmonary septum into the aortic and pulmonary semilunar valves. (C) In the final stages of outflow septation, the proximal part of the cardiac outflow tract (conus) is septated by the union of the conal cushions along a seam of cardiac neural crest cells. a, aorta, or aortic side; p, pulmonary trunk, or pulmonary side; c, conus cordis; t, truncus arteriosus; ivc, primary interventricular connection; rv, right ventricle; lv, left ventricle; pi, pulmonary infundibulum; dr, dorsal right truncal cushion; dl, dorsal left truncal cushion; vc, ventral cushion; r3, 4, 6, right third, fourth and sixth arch arteries; l3, 4, 6, left third, fourth and sixth arch arteries; ravc, right atrioventricular canal; lavc, left atrioventricular canal.

sheath is located at the junction of the distal outflow tract with the aortic sac.[66] Neural crest-derived mesenchyme condenses around the persisting aortic arch arteries and a population from each side grows into the distal outflow tract as two columns between the myocardium and endocardium. These columns are located in the distal outflow cushions. The two columns are connected across the midline dorsal to the aortic sac forming an ∩-shaped structure. The crosspiece of the ∩ bulges into the dorsal wall of the aortic sac between arch arteries 4 and 6.[79] The

legs of the ∩ are pulled toward the proximal outflow,[66] or contribute cells to the crosspiece to lengthen it toward the proximal outflow tract,[79] leaving a septum dividing the distal outflow tract (Figure 2.6). The condensed mesenchyme of the ∩ does not extend into the proximal outflow tract (conus). However, groups of neural crest cells lie under the endocardium of the endocardial cushions in the proximal outflow tract. Perhaps because the proximal cushions are becoming populated by myocardial cells (myocardialization), the cushions bulge into the lumen of the proximal outflow, causing the endocardium from each side to appose.[107] The endocardium breaks down, leaving a seam of neural crest-derived mesenchyme.[79,103,107] The remaining window in the ventricular septum is closed by growth of the AV endocardial cushions and ventricular septum, as discussed previously, and proximal outflow septum. Through the process of septation, the outflow tract is shortened and incorporated into the base of the heart, bringing the arterial valves to lie approximately at the level of the AV valves, with the aortic valve nestled between the mitral and tricuspid valves.

Semilunar and atrioventricular valves

The semilunar and AV valves are produced primarily from the endocardial cushion tissue formed in the tubular heart. The AV valves are formed when the AV cushions form prevalvular leaflets by delamination of ventricular myocardium underneath the cushion tissue.[104] This myocardium disappears, resulting in fibrous, nonmuscular valve leaflets. However, the original connections of the myocardium to the ventricular wall are remodeled into the papillary muscles and chordae tendinae.

The semilunar valves are sculpted from the remaining cushion tissue in the region of the truncus, which has now been incorporated into the base of the heart as the right ventricular infundibulum.[79] Epidermal growth factor receptor is required for normal semilunar, but not atrioventricular, valve development. In the absence of a functional receptor, the semilunar valves show fibrous hypertrophy.[105]

References

1. Rosenquist GC, DeHaan RL. Migration of precardiac cells in the chick embryo: a radioautographic study. Carnegie Institute, Washington, Publication 625. Contrib Embryol 1996;38:111–121.
2. Raffin M, Leong LM, Rones MS, Sparrow D, Mohun T, Mercola M. Subdivision of the cardiac Nkx2.5 expression domain into myogenic and nonmyogenic compartments. Dev Biol 2000;218:327–340.
3. Linask KK, Lash JW. Precardiac cell migration: fibronectin localization at mesoderm–endoderm interface during directional movement. Dev Biol 1986;114:87–101.
4. Cohen-Gould L, Mikawa T. The fate diversity of mesodermal cells within the heart field during chicken early embryogenesis. Dev Biol 1996;177:265–273.
5. Noden DM. Origins and patterning of avian outflow tract endocardium. Development 1991;111:867–876.
6. Suzuki HR, Solursh M, Baldwin HS. Relationship between fibronectin expression during gastrulation and heart formation in the rat embryo. Dev Dyn 1995;204:259–277.
7. Hurle JM, Ojeda JL. Cardiac jelly arrangement during the formation of the tubular heart of the chick embryo. Acta Anat 1977;98:444–445.
8. Hiruma T, Hirakow R. Epicardium formation of chick embryonic heart: computer-aided reconstruction, scanning, and transmission electron microscopic studies. Am J Anat 1989;184:129–138.
9. Kirby ML, Gale TF, Stewart DE. Neural crest cells contribute to aorticopulmonary septation. Science 1983;220:1059–1061.
10. Sohal GS, Ali MM, Ali AA, Dai D. Ventrally emigrating neural tube cells differentiate into heart muscle. Biochem Biophys Res Commun 1999;27:601–604.
11. Tam PPL, Parameswaran M, Kinder SJ, Weinberger RP. The allocation of epiblast cells to the embryonic heart and other mesodermal lineages: the role of ingression and tissue movement during gastrulation. Development 1997;124:1631–1642.
12. Antin RB, Taylor RG, Yatskievych T. Precardiac mesoderm is specified during gastrulation in quail. Dev Dyn 1994;200:144–154.
13. Schultheiss TM, Xydas S, Lassar AB. Induction of avian cardiac myogenesis by anterior endoderm. Development 1995;121:4203–4214.
14. Sugi Y, Lough J. Anterior endoderm is a specific effector of terminal cardiac myocyte differentiation of cells from the embryonic heart forming region. Dev Dyn 1994;100:155–162.
15. Antin PB, Yatskievych T, Dominguez JL, Chieffi P. Regulation of avian precardiac mesoderm development by insulin and insulin-like growth factors. J Cell Physiol 1996;168:42–50.
16. Nascone N, Mercola M. An inductive role for the endoderm in *Xenopus* cardiogenesis. Development 1995;121:515–523.
17. Lough J, Sugi Y. Endoderm and heart development. Dev Dyn 2000;217:327–342.
18. Jacobson AG. Influences of ectoderm and endoderm on heart differentiation in the newt. Dev Biol 1960;2:138–154.
19. Goldstein AM, Fishman MC. Notochord regulates cardiac lineage in zebrafish embryos. Dev Biol 1998;201:247–252.
20. Saga Y, Miyagawa-Tomita S, Takagi A, Kitajima S, Miyazaki J, Inoue T. MesP1 is expressed in the heart precursor cells and required for the formation of a single heart tube. Development 1999;126:3437–3447.
21. Linask KK. N-Cadherin localization in early heart development and polar expression of Na^+, K^+-ATPase, and integrin during pericardial coelom formation and epithelialization of the differentiating myocardium. Dev Biol 1992;151:213–224.
22. Linask KK, Knudsen KA, Gui YH. N-cadherin–catenin interaction: necessary component of cardiac cell compartmentalization during early vertebrate heart development. Dev Biol 1997;185:148–164.

23. Jiang YM, Tarzami S, Burch JBE, Evans T. Common role for each of the cGATA-4/5/6 genes in the regulation of cardiac morphogenesis. Dev Genet 1998;22:263–277.

24. Morrisey EE, Ip HS, Tang ZH, Lu MM, Parmacek MS. GATA-5: a transcriptional activator expressed in a novel temporally and spatially-restricted pattern during embryonic development. Dev Biol 1997;183:21–36.

25. Price RL, Chintanowonges C, Shiraishi I, Borg TK, Terracio L. Local and regional variations in myofibrillar patterns in looping rat hearts. Anat Rec 1996;245:83–93.

26. Sedmera D, Pexieder T, Hu N, Clark EB. Developmental changes in the myocardial architecture of the chick. Anat Rec 1997;248:421–432.

27. Gassmann M, Casagranda F, Orioli D et al. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 1995;378:390–394.

28. Lee K-F, Simon H, Chen H, Bates B, Hung M-C, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 1995;378:394–398.

29. Moorman AFM, de Jong F, Denyn MMFJ, Lamers WH. Development of the cardiac conduction system. Circ Res 1998;82:629–644.

30. Cheng G, Litchenberg WH, Cole GJ, Mikawa T, Thompson RP, Gourdie RG. Development of the cardiac conduction system involves recruitment within a multipotent cardiomyogenic lineage. Development 1999;126:5041–5049.

31. Gourdie RG, Mima T, Thompson RP, Mikawa T. Terminal diversification of the myocyte lineage generates Purkinje fibers of the cardiac conduction system. Development 1995;121:1423–1431.

32. Hyer J, Johansen M, Prasad A et al. Induction of Purkinje fiber differentiation by coronary arterialization. Proc Natl Acad Sci USA 1999;96:13214–13218.

33. de Groot IJM, Sanders E, Visser SD et al. Isomyosin expression in developing chicken atria: a marker for the development of conductive tissue? Anat Embryol 1987;176:515–523.

34. Lamers WH, te Kortschot A, Los JA, Moorman AFM. Acetylcholinesterase in prenatal rat heart: a marker for the early development of the cardiac conductive tissue? Anat Rec 1987;217:361–370.

35. Chuck ET, Watanabe M. Differential expression of PSA-NCAM and HNK-1 epitopes in the developing cardiac conduction system of the chick. Dev Dyn 1997;209:182–195.

36. Blom NA, Gittenberger-de Groot AC, DeRuiter MC, Poelmann RE, Mentink MMT, Ottenkamp J. Development of the cardiac conduction tissue in human embryos using HNK-1 antigen expression—possible relevance for understanding of abnormal atrial automaticity. Circulation 1999;99:800–806.

37. Hiltunen JO, Arumäe U, Moshnyakov M, Saarma M. Expression of mRNAs for neurotrophins and their receptors in developing rat heart. Circ Res 1996;79:930–939.

38. Sugi Y, Markwald RR. Formation and early morphogenesis of endocardial endothelial precursor cells and the role of endoderm. Dev Biol 1996;175:66–83.

39. Coffin DJ, Poole TJ. Embryonic vascular development: immunohistochemical identification of the origin and subsequent morphogenesis of the major vessel primordia in quail embryos. Development 1988;102:735–748.

40. Reaume AG, Conlon RA, Zirngibl R, Yamaguchi TP, Rossant J. Expression analysis of a Notch homologue in the mouse embryo. Dev Biol 1992;154:377–387.

41. Christ B, Schmidt C, Huang RJ, Wilting J, Brand-Saberi B. Segmentation of the vertebrate body. Anat Embryol 1998;197:1–8.

42. Nemer G, Bronchain O, Nemer M. Expression and regulation of a family of gata transcription factors in the mammalian heart. Mol Biol Cell 1995;6:419a.

43. Shalaby F, Rossant J, Yamaguchi TP et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995;376:62–66.

44. Shalaby F, Ho J, Stanford WL et al. A requirement for Flk1 in primitive and definitive hematopoiesis and vasculogenesis. Cell 1997;89:981–990.

45. Dumont DJ, Fong F-H, Puri MC, Gradwohl G, Alitalo K, Breitman ML. Vascularization of the mouse embryo: a study of *flk-2*, *tek*, *tie*, and vascular endothelial growth factor expression during development. Dev Dyn 1995;203:80–92.

46. Yamaguchi TP, Dumont DJ, Conlon RA, Breitman ML, Rossant J. *flk-1*, and *flt*-related receptor tyrosine kinase is an early marker for endothelial cell precursors. Development 1993;118:489–498.

47. Ferrara N, Carver-Moore K, Chan H et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996;380:439–442.

48. Carmeliet P, Ferreira V, Brier G et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435–439.

49. Beddington RS, Robertson EJ. Anterior patterning in mouse. Trends Genet 1998;14:277–284.

50. Yatskievych TA, Pascoe S, Antin PB. Expression of the homeobox gene *Hex* during early stages of chick embryo development. Mech Dev 1999;80:107–109.

51. Baldwin HS, Buck CA. Integrins and other cell adhesion molecules in cardiac development. Dev Biol 1994;121:220–236.

52. Hierck BP, Gittenberger-deGroot AC, van Iperen L, Brouwer A, Poelmann RE. Expression of the β4 integrin subunit in the mouse heart during embryonic development: retinoic acid advances β4 expression. Dev Dyn 1996;207:89–103.

53. Qu R, Silver MM, Letarte M. Distribution of endoglin in early human development reveals high levels on endocardial cushion tissue mesenchyme during valve formation. Cell Tissue Res 1998;292:333–343.

54. Vincent EB, Runyan RB, Weeks DL. Production of the transforming growth factor-beta binding protein endoglin is regulated during chick heart development. Dev Dyn 1998;213:237–247.

55. Moss JB, Xavier-Neto J, Shapiro MD et al. Dynamic patterns of retinoic acid synthesis and response in the developing mammalian heart. Dev Biol 1998;199:55–71.

56. Boyer AS, Ayerinskas II, Vincent EB, McKinney LA, Weeks DL, Runyan RB. TGFβ2 and TGFβ3 have separate and sequential activities during epithelial–mesenchymal cell transformation in the embryonic heart. Dev Biol 1999;208:530–545.

57. Wunsch AM, Little CD, Markwald RR. Cardiac endothelial heterogeneity defines valvular development as demonstrated by the diverse expression of JB3, an antigen of the endocardial cushion tissue. Dev Biol 1994;165:585–601.

58. Mjaatvedt CH, Yamamura H, Capehart AA, Turner D, Markwald RR. The *Cspg2* gene, disrupted in the *hdf* mutant, is required for right cardiac chamber and endocardial cushion formation. Dev Biol 1998;202:56–66.

59. Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. Circ Res 1995;77:1–6. Review.

60. Markwald R, Eisenberg C, Eisenberg L, Trusk T, Sugi Y. Epithelial–mesenchymal transformations in early avian heart development. Acta Anat 1996;156:173–186.

61. Erikson SL, O'Shea KS, Ghaboosi N et al. ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2- and heregulin-deficient mice. Development 1997;124: 4999–5011.

62. Yamagishi T, Nakajima Y, Sampath TK, Miyazono K, Nakamura H. Bone morphogenetic protein 2 acts synergistically with transforming growth factor β3 in endothelial–mesenchymal cell transformation during chick heart development. Ann NY Acad Sci 1998;857:276–278.

63. Rezaee M, Isokawa K, Halligan N, Markwald RR, Krug EL. Identification of an extracellular 130-kDa protein involved in early cardiac morphogenesis. J Biol Chem 1993;268:14404–14411.

64. Boyer AS, Erickson CP, Runyan RB. Epithelial–mesenchymal transformation in the embryonic heart is mediated through distinct pertussis toxin-sensitive and TGFβ signal transduction mechanisms. Dev Dyn 1999;214:81–91.

65. Galvin KM, Donovan MJ, Lynch CA et al. A role for Smad6 in development and homeostasis of the cardiovascular system. Nature Genet 2000;24:171–174.

66. Thompson RP, Fitzharris TP. Morphogenesis of the truncus arteriosus of the chick embryo heart: the formation and migration of mesenchymal tissue. Am J Anat 1979;154:545–556.

67. Virágh SZ, Challice CE. The origin of the epicardium and the embryonic myocardial circulation in the mouse. Anat Rec 1981;201:157–168.

68. Poelmann RE, Gittenberger-de Groot AC, Mentink MMT, Bökenkamp R, Hogers B. Development of the cardiac coronary vascular endothelium, studied with antiendothelial antibodies, in chicken–quail chimeras. Circ Res 1993;73:559–568.

69. Webb S, Brown NA, Anderson RH. Formation of the atrioventricular septal structures in the normal mouse. Circ Res 1998;82:645–656.

70. Perez-Pomares JM, Macias D, Garcia-Garrido L, Munoz-Chapuli R. The origin of the subepicardial mesenchyme in the avian embryo: an immunohistochemical and quail–chick chimera study. Dev Biol 1998;200:57–68.

71. Dettman RW, Denetclaw W Jr, Ordahl CP, Bristow J. Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intermyocardial fibroblasts in the avian heart. Dev Biol 1998;193:169–181.

72. Gittenberger-de Groot AC, Peeters MPFM, Mentink MMT, Gourdie RG, Poelmann RE. Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. Circ Res 1998;82:1043–1052.

73. Waldo KL, Willner W, Kirby ML. Origin of the proximal coronary artery stem and a review of ventricular vascularization in the chick embryo. Am J Anat 1990;188:109–120.

74. Bogers AJJC, Gittenberger-de Groot AC, Poelmann RE, Huysmans HA. Development of the origin of the coronary arteries, a matter of ingrowth or outgrowth? Anat Embryol 1989;180:437–441.

75. Hood LC, Rosenquist TH. Coronary artery development in the chick: origin and deployment of smooth muscle cells, and the effects of neural crest ablation. Anat Rec 1992;234:291–300.

76. Waldo KL, Kumiski D, Kirby ML. Cardiac neural crest is essential for the persistence rather than the formation of an arch artery. Dev Dyn 1996;205:281–292.

77. Folkman J, D'Amore PA. Blood vessel formation: what is its molecular basis? Cell 1996;87:1153–1155.

78. Le Lièvre CS, Le Douarin NM. Mesenchymal derivatives of the neural crest. Analysis of chimaeric quail and chick embryos. J Embryol Exp Morphol 1975;34:125–154.

79. Waldo KL, Miyagawa-Tomita S, Kumiski D, Kirby ML. Cardiac neural crest cells provide new insight into septation of the outflow tract: aortic sac to ventricular septal closure. Dev Biol 1998;196: 129–144.

80. Jiang X, Rowitch X, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cardiac neural crest. Development 2000;127: 1607–1616.

81. Creazzo TL, Godt RE, Leatherbury L, Conway SJ, Kirby ML. Role of cardiac neural crest cells in cardiovascular development. Ann Rev Physiol 1998;60:267–286.

82. Kirby ML, Waldo KL. Neural crest and cardiovascular patterning. Circ Res 1995;77:211–215.

83. Tomita H, Connuck DM, Leatherbury L, Kirby ML. Relation of early hemodynamic changes to final cardiac phenotype and survival after neural crest ablation in chick embryos. Circulation 1991;84:1289–1295.

84. Creazzo TL. Reduced "L" type calcium current in the embryonic chick heart with persistent truncus arteriosus. Circ Res 1990;66:1491–1498.

85. Nosek TM, Fogaca RTH, Hatcher CJ, Brotto MAP, Godt RE. Effect of cardiac neural crest ablation on contractile force and calcium uptake and release in chick heart. Am J Physiol Heart Circ Physiol 1997;273:H1464–H1471.

86. Waldo KL, Zdanowicz M, Burch J et al. A novel role for cardiac neural crest in heart development. J Clin Invest 1999;103:1499–1507.

87. de la Cruz MV, Markwald RR, eds. Living Morphogenesis of the Heart. Boston, Birkhaüser, 1998.

88. Arguello C, de la Cruz MV, Gomez CS. Experimental study of the formation of the heart tube in the chick embryo. J Embryol Exp Morphol 1975;33:1–11.

89. Yutzey KE, Rhee JT, Bader D. Expression of the atrial-specific myosin heavy chain AMHC1 and the establishment of anteroposterior polarity in the developing chicken heart. Development 1994;120:871–883.

90. Xavier-Neto J, Neville CM, Shapiro MD, et al. A retinoic acid-inducible transgenic marker of sino-atrial development in the mouse heart. Development 1999;126:2677–2687.

91. Bao ZZ, Bruneau BG, Seidman JG et al. Regulation of chamber-specific gene expression in the developing heart by Irx4. Science 1999;283:1161–1164.

92. Reecy JM, Li XY, Yamada M et al. Identification of upstream regulatory regions in the heart-expressed homeobox gene *Nkx2-5*. Development 1999;126:839–849.

93. Lien CL, Wu CZ, Mercer B, Webb R, Richardson JA, Olson EN. Control of early cardiac-specific transcription of *Nkx2-5* by a GATA-dependent enhancer. Development 1999;126:75–84.

94. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nature Genet 1997;16:154–160.

95. Henderson DJ, Copp AJ. Versican expression is associated with chamber specification, septation, and valvulogenesis in the developing mouse heart. Circ Res 1998;83:523–532.

96. Van Mierop LHS. Anatomy and embryology of the right ventricle. In: The Heart: International Academy of Pathology, Monograph No. 15. Baltimore: Williams and Wilkins, 1974:1–16.

97. Potocki L, Abuelo DN, Oyer CE. Cardiac malformation in two infants with hypochondrogenesis. Am J Med Genet 1995;59:295–299.

98. Hyett J, Moscoso G, Nicolaides K. Abnormalities of the heart and great arteries in first trimester chromosomally abnormal fetuses. Am J Med Genet 1997;69:207–216.

99. Basson CT, Bachinsky DR, Lin RC et al. Mutations in human cause limb and cardiac malformation in Holt–Oram syndrome. Nature Genet 1997;15:30–34.

100. Li QY, Newbury-Ecob RA, Terrett JA et al. Holt–Oram syndrome is caused by mutations in *TBX5*, a member of the *Brachyury (T)* gene family. Nature Genet 1997;15:21–29.

101. Van Mierop LHS, Kutsche LM. Development of the ventricular septum of the heart. Heart Vessels 1985;1:114–119.

102. de la Cruz MV, Moreno-Rodriguez R. Embryological development of the apical trabeculated region of both ventricles. The contribution of the primitive interventricular septum in the ventricular septation. In: Living Morphogenesis of the Heart. MV de la Cruz, RR Markwald, eds. Sinauer, 1998.

103. Waldo KL, Lo CW, Kirby ML. Connexin 43 expression reflects neural crest patterns during cardiovascular development. Dev Biol 1999;208:307–323.

104. Oosthoek PW, Wenink ACG, Vrolijk BCM et al. Development of the atrioventricular valve tension apparatus in the human heart. Anat Embryol 1998;198:317–329.

105. Chen BB, Bronson RT, Klaman LD et al. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nature Genet 2000;24:296–299.

106. Ehrman LA, Yutzey KE. Lack of regulation in the heart forming region of avian embryos. Dev Biol 1999;207:163–175.

107. Van den Hoff MJB, Moorman AFM, Ruijter JM et al. Myocardialization of the cardiac outflow tract. Dev Biol 1999;212:477–490.