Cardiac Septation
A Late Contribution of the Embryonic Primary Myocardium to Heart Morphogenesis

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Abstract—Heart morphogenesis comprises 2 major consecutive steps, viz. chamber formation followed by septation. Septation is the remodeling of the heart from a single-channel peristaltic pump to a dual-channel, synchronously contracting device with 1-way valves. In the human heart, septation occurs between 4 and 7 weeks of development. Cardiac looping and chamber formation bring the contributing structures into position to engage in septation. Cardiomyocytes that participate in chamber formation do not materially contribute to septation. The (re)discovery of the role of extracardiac mesenchymal tissue in atrioventricular septation, the appreciation that the formation of the right atrioventricular connection is more than a mere rightward expansion of the atrioventricular canal, the awareness that myocardium originating from the so-called anterior heart field regresses after its function as outflow-tract sphincter ceases, and the recent finding that the myocardialized proximal portion of the outflow-tract septum becomes the supraventricular crest have all significantly enhanced our understanding of the morphogenetic processes that contribute to septation. The bifurcation of the ventricular conduction system is the landmark that separates the contribution of the atrioventricular cushions and the outflow-tract ridges to septation and that divides the muscular ventricular septum in inlet, trabecular, and outlet portions. (Circ Res. 2002;91:93-103.)

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emphasis on the developing human heart and on structures developed in our group and elsewhere over the last 10 years. The account will focus on the mammalian heart, with an account of our understanding of cardiac septation as it has critically modified mice. In the following, we will give an application to later development, including much of septation, the atrial spine. 1 indicates inferior (caudal) atrioventricular cushion; 2, superior (cranial) atrioventricular cushion; 3, parietal outflow-tract ridge; 4, septal outflow-tract ridge; AS, atrial spine with spur (S) on leading edge of primary atrial septum (PAS); 6, body of atrium; 7, primary ring; PIF, primary interatrial foramen; RA, right appendage; LA, left appendage; AVC, atrioventricular canal; LV, embryonic left ventricle; RV, embryonic right ventricle; and OFT, outflow tract.

the remaining regions. These latter regions, that is, the structures of the atrial midline, the atrioventricular junction, the inner curvature of the heart loop, and the outflow tract, temporally retain the properties of the primary myocardium of the embryonic heart tube. Intriguingly, the morphogenetic events that lead to cardiac septation are largely confined to these less advanced structures, implying that myocytes that participate in the first step (chamber formation), do not materially contribute to the second step (septation).

Description cannot provide conclusive evidence in favor of mechanistic hypotheses. Morphological observations, nevertheless, often initiate such hypotheses. Indeed, flawed descriptions or interpretations of morphology have generated useless hypotheses and, hence, hindered progress. Cardiac embryology has certainly been a case in point. Fortunately, the implementation of new techniques has facilitated interpretation and, hence, changed the appreciation for embryology. In this respect, in vivo labeling of cells is certainly the most definitive technique for establishing the origin and fate of major components of the heart (summarized in De la Cruz and Markwald). Unfortunately, the accuracy of this technique has its limitations, in particular when the label cannot be positioned on an unambiguous landmark. In addition, its application to later development, including much of septation, is often limited by poor accessibility of the relevant structures. Despite their inherent shortcomings, phenotypic markers have, therefore, proven to be very useful for delineating structures that participate in septation and for tracking their fate in consecutive stages of development. Furthermore, the morphogenetic models that were deduced from these data can now often be tested for compatibility with extant congenital malformations, including those resulting from genetically modified mice. In the following, we will give an account of our understanding of cardiac septation as it has developed in our group and elsewhere over the last 10 years. The account will focus on the mammalian heart, with an emphasis on the developing human heart and on structures that are considered to be prime movers in the morphogenetic processes underlying septation. Carnegie stages are assigned to all pertinent events, which may facilitate future reading, because ultrasound data may change the longstanding relation between developmental age and Carnegie stages, and should facilitate the comparison with mouse or rat development. Cranial, caudal, dorsal, and ventral will be used as indicators of orientation, with the apex of the heart always pointing ventrally. It should be noted that, in the postnatal human heart, these terms correspond to superior, inferior, posterior, and anterior, respectively. For description of the atrioventricular endocardial cushions, we have retained the commonly used terms “superior” and “inferior.”

Septation of the Atria

Left and right appendages develop from the caudal part of the heart-forming regions. This part of the heart-forming regions does not participate in looping but, instead, retains its left and right identity. Indeed, sidedness, as revealed by the different morphology of left and right appendages, is a determining factor for atrial development.

The left and right atria develop symmetrically up to the 11th stage (~22 days), when their caudal continuation into both sinus horns begins to move rightwards, coincident with rapid growth of the atria and the right sinus horn, whereas growth of the left sinus horn falls behind. The sinuatrial junction as such becomes identifiable as the right-sided sinuatrial foramen during the 12th stage (~26 days). The protrusion of the sinuatrial foramen into the lumen of the atrium generates the so-called venous valves. The lower, caudal portion of the venous valves is a true fold, whereas the upper, cranial portion is a single-layered structure, suggesting development by proliferation. The upper commissure of the venous valves continues as the septum spurium in the roof of the right atrium, whereas the ventricular ends of the valves insert near the midline on the so-called
“atrial spine.” The venous valves therefore occupy a plane that intersects the sagittal plane of the atrium at a 45° angle.

During the 13th stage (~28 days of development), the pulmonary vein is seen to develop from an endothelial evagination into the dorsal mesocardium, just cranial to the “sinus septum” that marks the confluence of both sinus horns.13,16–19 Relative to the size of the heart, the dorsal mesocardium, that is, the attachment of the caudal portion of the heart tube to the dorsal body wall, is still a relatively large structure at this stage12a,20 and surrounds the orifice of the pulmonary vein as a rim that generates the typical pit-like appearance of the orifice of the pulmonary vein when inspected from the atrial cavity.17,18 Cranial to the pulmonary pit, the primary atrial septum develops as a crescent-shaped muscular septum that expands from the dorsal wall of the atrium toward the atroventricular canal in the 5th and 6th weeks (14th through 16th stages; Figure 1). The left-sided rim of the pulmonary pit is a transient structure, but the right-sided rim increases in size to become the so-called “spina vestibuli.”17,19,21–24 This structure, first described by His,21 owes its name to the Latin translation of the German word for atrium ("Vorhof") and should therefore be renamed the “atrial spine” in English texts. The body of this extracardiac tissue penetrates the atrium as a prong of mesenchyme on top of the inferior atroventricular endocardial cushion,17,21,24 whereas its cranial spur follows the developing primary atrial septum as the thin mesenchymal cap on its leading edge.15,23,24 The communication between the left and right atrium underneath the primary atrial septum (the “primary” foramen) closes in the second half of the 6th week (16th and 17th stages6,18,24) when the body of the atrial spine and the mesenchymal cap on the primary atrial septum merge and fuse with the superior endocardial cushion. Shortly thereafter, both atroventricular endocardial cushions begin to fuse, creating separate left and right atroventricular connections.25–27 Meanwhile, a number of fenestrations develop in the dorsal portion of the primary atrial septum to form a new interatrial communication, the “secondary” foramen.

There is some argument as to whether the pulmonary orifice lies ab initio within the confines of the atrium13,17,28 or is secondarily recruited from the sinus venosus.18,19 The argument centers on the question whether or not a temporarily recognizable (during the 12th stage only) shallow fold to the left of the pulmonary pit is an upward extension of the caudal sinusatrial fold. This temporary ridge is argued to be a sinus venosus structure, because it expresses HNK-1 in the chick,29 but has disappeared when the pulmonary vein acquires access to the left atrium.29 These arguments underscore that the initiation of pulmonary vein development is intimately linked with the development of asymmetry within the venous pole of the heart. Indeed, the dorsal mesocardium itself is highly polarized, because its left-sided epithelium strongly expresses the left-sided markers creatine kinase-B (CK-B; Figure 2)15 and Pitx-2,30 whereas its right-sided counterpart does not. Furthermore, the myocardium surrounding the developing pulmonary vein (“pulmonary myocardium”) is Pitx2-positive,11 indicating its left identity. The pulmonary vein is therefore a left-sided structure in the dorsal mesocardium, lying to the left of the atrial spine and on top of the inferior atroventricular cushion when it enters the atrial cavity.15,18

The present description of atrial septation differs from that in standard textbooks in the decisive role that is attributed to the atrial spine in septating the atrium and closing the primary foramen. The body of the spine is tightly attached to the inferior atroventricular cushion, from which it can only be distinguished immunohistochemically.15,24 There is still some argument whether the mesenchymal cap on the leading edge of the primary atrial septum is of extracardiac origin, as we claim based on its staining properties,15,24 or arises from a local epitheliomesenchymal transformation similar to that seen for the atroventricular cushions.31–33 Although the extent to which the spine penetrates the atrium is therefore still being disputed, there is general agreement that the expansion of the body of the spine over the endocardial cushions is instrumental for the closure of the primary
interatrial foramen. The importance of the atrial spine for atroventricular septation is also supported by data from mouse mutants, in which defective development of the atrial spine, including its spur, is associated with persistence of the foramen primum and the development of atrioventricular septal defects. In these mice, the atroventricular cushions almost always fuse.

Soon after closure of the primary foramen, the spine becomes muscular. Myocardialization starts near the sites of attachment of the primary atrial septum and the venous valves on the spine. In preseptation hearts, the sinusatrial fold and the pulmonary pit are adjacent structures in the caudal wall of the atrium. When the atrial spine expands into the atrium, the venous valves become anchored to the spine. Myocardium largely replaces the mesenchymal component of the spine, except in its center, where Todaro’s tendon forms. The primary atrial septum forms the flap valve of the oval foramen in the formed fetal heart, whereas the myocardIALIZED atrial spine forms the ventral and caudal rims of the oval fossa. The dorsal and cranial rims of the oval fossa (the “limbus”) are formed by the so-called secondary septum of the atrium, a structure that develops as an infolding of the dorsal atrial wall rather than as a septum between the entrance of the systemic and pulmonary veins. It should be emphasized that the structure, which develops coincident with the regression of the left venous valve, is difficult to identify in the embryo and only becomes a pronounced fold in the course of the second trimester of pregnancy.

The observation that the definitive atria appear to comprise 4 transcriptional domains, namely, the myocardium of the sinus venosus, the atrial appendages, the atrioventricular canal, and the dorsal mesocardium, suggests that these domains reflect tissue sources and, hence, the lineage of atrial myocardium. The domain of the dorsal mesocardium includes, in addition to the atrial spine, the components of the definitive interatrial septum and the myocardium surrounding the pulmonary veins. The dorsal fold that forms the secondary atrial septum develops at the boundary of the expression of the left-sided markers CK-B15 and Pitx2, and delineates the original midline of the atria. This finding implies that the primary atrial septum is a left-sided structure. In agreement with this hypothesis, the primary atrial septum abundantly expresses the left-sided markers CK-B15 and Pitx2.

Septation of the Atrioventricular Junction

Because the atroventricular canal and the ventricles are part of the heart tube that undergoes rightward looping, the originally left-sided portion of the heart tube acquires a ventral position and the right-sided portion a dorsal position. Hence, the left and right ventricles are each made up of contributions of both the left- and right-sided heart fields. Although this lack of a “simple” bilateral symmetry in the atroventricular canal and ventricles has been known for more than 40 years, its consequences for the morphogenesis of this region during septation have only recently been acknowledged. Another complicating development is the more pronounced growth in the dextro-caudal portion of the atroventricular canal and the adjacent inlet portion of the right ventricle (both derivatives of the right-sided heart field) relative to that of the left-sided counterparts in the 5th and 6th weeks. As a result, a generally accepted account of the morphological changes that accompany the remodeling of the atroventricular junction during septation is still lacking. The present account is largely based on the phenotypical identification of structures and the interpretation of the spectrum of malformations that is seen in pertinent mutant mice.

Up to the middle of the 6th week (15th stage), the atroventricular canal is largely positioned above the left ventricle. Because a large “primary” interventricular foramen exists and because the ventricle remains relaxed until atrial contraction is completed, atrial blood can pass directly to the right ventricle. However, after separate atroventricular connections have been established, the right atrium must be in direct contact with the right ventricle. The necessary remodeling of the right atrioventricular junction proceeds coincident with the fusion of the atroventricular cushions late in the 6th and in the 7th week. To understand this remodeling process, one has to keep in mind that growth in the preseptational heart is strongest in the outer curvature and virtually absent in the inner curvature. As a result, the “junctural” myocardium between atria and ventricles and that between embryonic left and right ventricles share the myocardial wall in the short inner curvature (Figure 1). In the human heart, the myocardium surrounding the atroventricular canal is distinct from that of the atria and ventricles in that it does not express creatine kinase M (CK-M), whereas the myocardium surrounding the primary interventricular foramen distinguishes itself from that of the ventricles in that it carries the sulfoglucuronol-carbohydrate epitope that is recognized by the Gln2/HNK-1/Leu7 antibodies. Because this latter myocardium surrounds the primary interventricular foramen and is the precursor of the ventricular conduction system, it was labeled the “primary ring.” The phenotype of the portion of the myocardium in the lesser curvature that is shared by the atroventricular canal and the primary ring is therefore both CK-M-negative and sulfoglucuronol-carbohydrate-positive.

Because growth in the atroventricular canal is more pronounced on the atrial than on the ventricular side, and on the dextro-caudal (that is, originally right) side than on the opposite side, the atroventricular canal becomes an asymmetric, funnel-shaped structure. This asymmetric growth in the atroventricular-canal region also comprises the part of the inner curvature that the atroventricular canal shares with the primary ring, as well as the adjacent part of the right ventricle. We previously dubbed the right-ventricular structure that forms as a result of the expansion of the atroventricular canal within the confines of the primary ring the “tricuspid gully.” A gully that is similar to, but substantially smaller than the tricuspid gully, is present at the junction of the atroventricular canal with the left ventricle, again emphasizing the pronounced left-right asymmetry in growth during septation.

At 5 weeks, a muscular septum is identifiable between the trabecular portions of both ventricles. We define this portion of the muscular septum as its “middle” portion. The caudal portion of the muscular septum, that is, the portion supporting the developing atroventricular canal and the inferior endocardial cushion, develops later, coincident with the changing...
configuration of the atrioventricular canal, as can be demonstrated by the topography of the emerging ventricular conduction system: at 5 weeks (15th stage), the bundle branches still occupy the caudal wall of both ventricles, whereas at 6 weeks (17th stage), these structures, including the atrioventricular bundle of His, are found on the crest of the caudal portion of the muscular ventricular septum.\textsuperscript{24,40} Indeed, casts of the cardiac cavities confirm the rapid development of the caudal portion of the septum between 5.5 and 6 weeks (15th through 17th stages).\textsuperscript{14} We define this caudal portion of the muscular ventricular septum as its “inlet” portion.

The tricuspid gully is mounted medially on the rim of the inlet portion of the ventricular septum. Interestingly, this portion of the septum is bent leftward relative to its middle, trabecular portion (Figures 3A through 3D), the bend thus marking the boundary between both components. As argued above, the pronounced growth of the dextro-caudal portion of the atrioventricular canal, the tricuspid gully, and the inlet portion of the muscular ventricular septum appear to be linked events. Indeed, mice with atrioventricular septal defect but also fail to develop the tricuspid gully and lack a right atrium and a left-sided position of the atrioventricular canal, not only suffer from a persisting primary foramen of the membranous septum,\textsuperscript{14} but also the atrioventricular endocardial cushions form the atrial surface of the atrioventricular valves\textsuperscript{49} and, at their site of fusion, the atrioventricular portion of the membranous septum of the heart. The interventricular portion of the membranous develops much later, in the course of the last trimester of pregnancy, after the delamination of the medial leaflet of the tricuspid valve has reached the top of the muscular ventricular septum.\textsuperscript{30} This delamination occurs within the myocardium, so that the ventricular surface of the leaflets is covered by coarse fibrous tissue stemming from the myocardium, whereas their atrial surface is covered with the smooth derivative of the atrioventricular cushions.\textsuperscript{40} The plane of delamination suggests that the myocardium of the tricuspid gully delaminates from that of the inlet portion of the muscular ventricular septum. Because trabeculae are absent, the surface of the right ventricular inlet underneath the medial tricuspid leaflet is said to be smooth. Interestingly, the plane of delamination of the medial tricuspid leaflet changes smoothly into that of the right atrioventricular sulcus, most likely because their overlying structures, the tricuspid gully and the right-sided atrial vestibule, respectively, both owe their development to the selectively right-sided growth of the atrioventricular-canal region during septation.\textsuperscript{24} The deepening of the right atrioventricular sulcus is therefore also an inherent part of cardiac septation.

This account of atrioventricular septation\textsuperscript{24} differs from existing descriptions in identifying the originally right-sided heart anlage as a major contributor to growth in the atrioventricular region and the source of tissue forming the septating structures. Although this conclusion was already suggested by in vivo marking experiments,\textsuperscript{5} very few studies have detailed the growth of the atrioventricular canal myocardium during the septation period. In contrast, the embryological origin of the so-called “inlet” to the right ventricle, that is, the portion of the right ventricle that contains the atrioventricular valve and its tension apparatus, has long been a hotly debated issue. Two hypotheses have been put forward to explain the development of a connection between the right atrium and the right ventricle that is as wide as that between the left atrium and the left ventricle: either the caudal portion of the muscular ventricular septum grows toward the middle of the atrioventricular canal before atrioventricular expansion occurs\textsuperscript{51,52} or the atrioventricular canal expands, relative to the muscular ventricular septum, to the right.\textsuperscript{51,53} The first hypothesis was mainly based on an account of aggregating trabeculae on the caudal wall of the left ventricle of a stage 12 (3.5 weeks) embryo.\textsuperscript{51,52} We (W.H.L., unpublished data, 1995) have reinspected the key specimen underscoring this hypothesis and have interpreted the trabecular aggregation as an artifact that was not seen in adjacent sections of the same embryo or in other embryos of the same stage. The alternative hypothesis requires a selective increase in the diameter of the atrioventricular canal during atrioventricular septation. Although the diameter of the atrioventricular connection more than doubles between 4.5 and 5.5 weeks of development (14th and 15th stages), it remains constant between 5.5 and 7 weeks (16th through 19th stage),\textsuperscript{53} that is, during septation. These data therefore strongly support our finding that the right atrioventricular connection expands in the 7th week by the formation of fenestrations in the floor of the tricuspid gully.
The “posterior (ie, caudal) smooth” or “sinus” septum on top of the “trabeculated” muscular septum53 appears to refer to the caudal or inlet component of the muscular ventricular septum that we discussed earlier. In particular, its angular orientation relative to the middle, trabecular portion of the muscular ventricular septum agrees with our observations. However, neither its origin nor its cranial boundary, which we put at the bifurcation of the bundle branches, was delineated. The development of the right-ventricular inlet from myocardium between the bundle of His medially and the right bundle branch or septomarginal trabeculation laterally has been hypothesized.54,55 The present integration of the development of the atrioventricular canal with that of the inlet portion of the ventricles into a single morphogenetic process has made it clear that malformations such as double inlet left ventricle, atrioventricular septal defects, tricuspid atresia, and Ebstein’s anomaly should be considered as the lasting consequences of a temporary arrest of normal development.24,35,41,56

**Septation of the Outflow Tract**

To understand septation in the outflow tract, a brief review of its natural history is useful. The outflow tract connects the embryonic right ventricle with the aortic sac and can first be recognized in the 2nd half of the 4th week (12th stage).14 In the 5th week of development (14th and 15th stages), the outflow tract rapidly increases in length, while the ventricles transform from peristaltoid to synchronously contracting compartments14,57 (cf, Argüélo et al58 and de Jong et al59). The long sleeve of slowly conducting myocardium with long-lasting contractions functions as a sphincter that prevents regurgitation during ventricular relaxation59 before the appearance of the arterial valves in the 7th week. During this period, the outflow tract can be divided into distinct proximal and distal portions, separated by a distinct bend. During contraction, the bend becomes accentuated, which may assist in avoiding regurgitation. Only after its function as a sphincter and its phenotypic properties as primary myocardium were revealed,59 the outflow tract became a developmental entity, its proximal (upstream) and distal portion corresponding to the “conus” and “truncus,” respectively.50 Because the terminology used to describe the respective parts of the outflow tract continues to be contentious, we will use the more descriptive terms proximal and distal portion.

The endocardial jelly within the outflow tract functions as the stuffer material of the outflow-tract sphincter. In the course of the 5th week, the cuff of the endocardial jelly transforms into distinct septal and parietal ridges.14 This transformation begins proximally and moves into the distal outflow tract when the 6th arch arteries appear at 4.5 weeks (14th stage).59 The septal ridge originates on the right side of the trabeculated muscular ventricular septum just cranial to the right bundle branch, whereas the parietal ridge originates where the outflow tract meets the right ventricle and right atrium in the inner curvature. Going from proximal to distal, endocardial ridges follow a rightward spiraling course that reflects, or at least follows, the rotational change in the outflow tract that accompanies the process of looping, but with a delay of 1 week. The sculpting of the ridges in the distal outflow tract is accompanied by, and perhaps results from, the penetration into them of neural crest–derived cells. This invasion of neural crest cells may also trigger the onset of fusion, from distal to proximal, of the ridges in the 6th week.39,61 The fused ridges thus form a spiraling septum that separates the outflow tract into a channel that connects to the 3rd and 4th arch arteries and a channel that connects to the 6th arch arteries.

This description differs from the currently used model in the role we attribute to the so-called aortopulmonary septum in establishing septation in the distal outflow tract. The prevailing view is that cells from the pharyngeal region migrate toward the outflow tract to form its distal mesenchymal or truncal portion. In conjunction, neural crest–derived mesenchyme is said to descend between the 4th and the 6th branchial arches to divide, as the aortopulmonary septum, the distal (mesenchymal or truncal) outflow tract into separate aortic and pulmonary channels.62-65 The neural crest–derived cells would further penetrate into the proximal outflow tract as “prongs” of condensed mesenchyme within the septal and parietal ridges.62-64 However, this description condenses the events that occur during the 5th and 6th weeks of development (14th through 17th stages) to such an extent that it becomes inaccurate.

The increase in length of the outflow tract during the 5th week is accompanied by distal expansion of the myocardium along its outer layer, so that myocardium covers the entire outflow tract up to its connection with the pharyngeal region by the end of this week (15th stage57,65; J.-S. Kim, S. Webb, A.F.M. Moorman, R.H. Anderson, W.H. Lamers, unpublished data, 2002). Similarly, the outflow tract in chicken embryos increases in length between Hamburger and Hamilton stages 12 and 21.66 There is good reason to believe that the (myocardial) cells forming this part of the outflow tract are derived from the pharyngeal region in both mammals and birds.67-70 This pharyngeal source of cardiomyocytes in the outflow tract is now called the “anterior” or “secondary” heart field.67,70 The phase of expansion of the myocardial outflow tract is followed by a rapid regression of the myocardium from its distal (truncal) portion toward the bend, again both in mammals and in birds57,71 (Kim et al, unpublished data, 2002). The cardiomyocytes at the distal boundary of the regressing myocardium show no signs of apoptosis, suggesting that they transdifferentiate into the cells forming the wall of the great arteries.57,71 This shortening of the myocardial portion of the outflow tract in the 6th week, and the coincident increase in the length of the nonmyocardial arterial trunks therefore occurs after the pharyngeal cells of the anterior heart field have contributed to the formation of the outflow tract. The abrupt and absolute shortening of the myocardial portion of the outflow tract has previously been described as the “absorption of the conus.”66,72,73 Nevertheless, the distal myocardial boundary at the junction of the outflow tract with the aortic sac as it is present at 5 weeks of development is usually considered to be identical with the position of the future arterial valves.62,72-75

The distal myocardial boundary of the outflow tract was therefore implicitly considered to be a fixed landmark for the position of the ventriculoarterial junction. Because the junction of the outflow tract with the 4th pair of branchial arches
lies cranial to that with the 6th pair, whereas the root of the aorta lies caudal to that of the pulmonary trunk, the apparent change in position of the aortic and pulmonary roots has been interpreted to result from rotation during septation.62,73 Such a rotation of the ventriculoarterial junction would require a compensatory downstream counterrotation. Instead, our analysis of outflow-tract development in rat and human embryos57 (Kim et al, unpublished data, 2002) shows that, concomitant with regression of the distal myocardial boundary, the spiraling segments of the aortic and pulmonary channels move from within to outside the myocardial heart. This finding reveals that the intrapericardial portion of the aorta and pulmonary trunk largely derives from the distal (truncal) portion of the outflow tract and forms separate channels due to fusion of the distal endocardial ridges. The observation that the root of the great arteries contains hardly any neural crest cells compared with the more distal parts61,76 underscores this conclusion. The aortopulmonary septum, therefore, does not descend from the aortic sac to the ventriculoarterial junction. Instead, it is limited to a small, transversely oriented wedge of tissue between the origins of the arteries supplying the 4th and 6th branchial arches.

The downward migration of the neural crest cells of the aortopulmonary septum is usually associated with formation of the prongs of condensed mesenchyme within the septal and parietal ridges.62-64 In agreement with the near absence of neural crest cells from the root of the aorta and pulmonary trunks, we were unable to find a direct continuity between the aortopulmonary septum and the prongs, suggesting that the neural crest cells migrate through, but do not persist in the distal outflow-tract ridges.76 At the 16th stage, the prongs fuse and form the characteristic midline whorl just proximal to the bend in the outflow tract57,62-64 (Kim et al, unpublished data, 2002). It is at this position that the valvar leaflets, still surrounded by a cuff of outflow-tract myocardium, form from the tissues of the endocardial ridges22,57,77 (Kim et al, unpublished data, 2002). The whorl and the proximal ends of the prongs that connect the whorl to the lateral myocardial wall64 mark the plane of separation between the aortic and pulmonary portions of the arterial valves. The aortic and pulmonary roots, including the arterial sinuses, with their valvar leaflets, develop in their entirety from the endocardial and intercalated ridges, without a significant contribution from neural crest-derived cells.57,61,76,78 In fact, the prominence of the neural crest–derived cells during the period of fusion and their disappearance after the ridges have fused76,79 suggest that their primary role is to assure proper fusion of the ridges.80

The onset of formation of the valves corresponds with the initiation of development of the coronary arteries. The myocardium that initially surrounds the arterial sinuses, including the roots of the coronary arteries57 (Kim et al, unpublished data, 2002), gradually disappears in the fetal period, the sinutubular junction still marking the distal boundary of this temporary myocardial cuff in the formed heart. The degenerate appearance of the myocytes just before their disappearance,57 coupled with the protracted course of the process, suggests that regression of myocardium from the proximal outflow tract is not a simple continuation of the initial regression from the distal outflow tract. Incomplete regression of this myocardium appears to be one of the causes of ventricular tachycardia.80 While the myocardial cuff slowly regresses, both arterial roots become separate structures that begin to move apart in the 2nd trimester. The disappearance of the myocardium that initially separates the developing left coronary leaflet of the aortic valve and the medial leaflet of the mitral valve is also part of this second wave of demyo-
cardialization,57,81 but the degree to which this latter myocardial regression is completed varies.82 Nevertheless, the left-sided predominance of this regressive development is striking, contrasting as it does with the increase in size of the myocardium of the subpulmonary infundibulum. The fusion of the proximal portion of the septal and parietal ridges at 7.5 weeks (21st stage) completes the septation of the outflow tract. At 6 weeks (17th stage), that is, well before fusion, the portion of the outflow-tract ridges below the arterial valves begins to myocardialize50,57,83,84 (Kim et al, unpublished data, 2002). The myocardialization continues after fusion, producing a muscular partition between the subaortic and subpulmonary vestibules that does not lie in line with the muscular ventricular septum. Instead, the muscular partition is supported exclusively by the developing right ventricle, its proximal boundary (in the formed heart) extending between the attachment of the medial papillary muscle on the ventricular septum and the lateral boundary of the supraventricular crest at the junction of the outflow tract with right ventricle and right atrium in the inner curvature. As a result, the right ventricle and the subaortic vestibule temporarily continue to communicate across the muscular ventricular septum behind, that is, caudal to the muscular partition. At this time, the atrioventricular cushions have already fused (see earlier paragraph). The premier factor that closes this remaining communication appears to be growth of the muscular partition (Kim et al, unpublished data, 2002). If the communication persists, as is seen in many hearts with ventricular septal defects, particularly tetralogy of Fallot and double outlet right ventricle, the muscular partition persists as an “outlet septum” between the subaortic and subpulmonary vestibules. If the communication is closed, as happens in normal development, it loses its septal position (see later paragraph).

A key to understanding morphogenesis in the proximal portion of the outflow tract is to appreciate its transformation from a tubular structure at 5 weeks to a wedge-shaped structure at 7 weeks of development. The asymmetric, right-sided development results from continued regression of myocardium on the left side of the outflow tract (see above) and continued growth in the corresponding right-sided part of the outflow tract. Likewise, the muscular partition between the subaortic and subpulmonary vestibules is cone-shaped with its medial apex (derived from the septal ridge) to the muscular ventricular septum and with its wide lateral base (derived from the parietal ridge) to the lateral wall of the right ventricle. The muscular partition between the subaortic and subpulmonary vestibules is characterized by a distinctive parallel orientation of its muscle fibers. Smooth muscle actin–positive cells may play a prominent role in determining the parallel course of the myocytes during their medial migration into the...
The unique parallel alignment of the myofibrils in the muscular partition remains identifiable in the postnatal heart and permits its delineation in the caudomedial wall of the outlet portion of the right ventricle (Figure 4). The transition from a compact, cone-shaped muscular partition to a long, relatively thin myocardial sheet that connects the arterial valves of the aorta and pulmonary trunk is a protracted event that begins at 8 weeks and is completed only in the course of the second trimester. The flattening of the muscular partition accentuates the offset between the leaflets of the pulmonary and aortic valves. Because the septal ridge is smallest and tethered to the muscular ventricular septum, the left-sided sinuses of the arterial valves, which derive from this ridge, stay closest together, whereas the right-sided sinuses, which derive from the bigger parietal ridge, move furthest apart. Consequently, the attachment of the right coronary cusp of the aortic valve projects on the upstream end of the supraventricular crest, whereas the corresponding cusp of the pulmonary valve forms the downstream boundary of the freestanding right-ventricular infundibulum. The muscular partition, therefore, continues to connect the arterial valves of the aorta with those of the pulmonary trunk, in doing so forming supraventricular crest and the caudomedial wall of the freestanding right-ventricular infundibulum. In the process, however, the muscular partition loses its position as a ventricular outlet septum, which it had in embryonic hearts.

The present account of outflow-tract development differs in 2 aspects from existing descriptions. First, we have explored the consequences of finding that the myocardium that originates from so-called anterior heart regresses after its function as the cuff of the outflow-tract sphincter ceases (Kim et al, unpublished data, 2002). This led us to appreciate that the proximal portion of the great arteries,
including the sinuses the arterial valves, belong to the embryonic outflow tract and, therefore, had a myocardial external lining when first formed in the developing heart. The bend between the proximal (conal) and distal (truncal) portions of the outflow tract can be traced to the sinus tubular junction in the great arterial trunks of postnatal hearts, showing that the arterial valves develop from the distal portion of the conus. Second, it was realized that the myocardialization of the proximal (conal) portion of the septum in the outflow tract should have important implications for the closure of the interventricular communication, this being the last step in septation. In contrast to the prevailing accounts of the final stages of septation (eg, van Mierop87 and Los88), we attribute significance to the growth displacement, well-accepted explanations of the development of double-outlet right ventricle and tetralogy, respectively, of the muscular septum that separates the subaortic and subpulmonary infundibula in completing septation. This explanation places the lack of sufficient growth or cranial displacement, well-accepted explanations of the development of double-outlet right ventricle and tetralogy, respectively, into perspective as causes of septation defects. Finally, the characteristic cytoarchitecture of the muscular septum allowed us to show how a septal structure in the outflow tract becomes the myocardial caudomedial wall of the freestanding right-ventricular infundibulum.

Our account shows that the muscular ventricular septum is formed from 3 components: the primary or trabecular component that develops from the septum separating the trabeculated cavities of the developing left and right ventricles; the inlet component that forms as part of the structures that bring about septation of the atrioventricular junction; and the outlet component that evolves from the structures that bring about septation in the proximal outflow tract. The bifurcation of the atrioventricular bundle into its bundle branches marks the boundary between the trabecular middle component and both the inlet and the outlet components of the muscular ventricular septum. The bifurcation of the His bundle also marks the divide between the contribution of the atrioventricular cushions and the outflow-tract ridges to septation, the atrioventricular cushions reaching to the bifurcation, and the endocardial ridges of the outflow tract starting distal to the right bundle branch.

An important difference between the atrioventricular cushions and the outflow-tract ridges is that the former are made up mostly of mesenchymal cells that are derived from the endocardium,89 with the extracardiac mesenchymal contribution to atrioventricular septation remaining confined to the atrial spine, whereas the outflow-tract ridges incorporate a substantial contribution of neural crest–derived mesenchymal cells.76,90,91 An additional, possibly associated, difference is that the atrioventricular cushions do not become myocardialized, at least in man and in rodents, this process being confined to the atrial spine. In contrast, the proximal portion of the endocardial ridges of the outflow tract does become myocardialized almost entirely to form the supraventricular crest and the caudal wall of the freestanding right-ventricular infundibulum (Kim et al, unpublished data, 2002).

Figure 4. Fate map of embryonic structures in the adult heart. Indicators of orientation as used in this review are as follows: Cr, cranial; Ca, caudal; D, dorsal; V, ventral; L, left; R, right. Color codes identifying the embryonic origin of adult structures are as follows: 1, sinus venosus; 2, primary atrial septum; 3, secondary atrial septum; 4, atrial spine; 5, left/right appendage; 6, atrioventricular canal; 7, superior endocardial cushion; 8, inferior endocardial cushion; 9, ventricular inlet/tricuspid gully; 10, left-lateral endocardial cushion; 11, outflow tract; 12, septal endocardial ridge; and 13, parietal endocardial ridge/right-lateral atrioventricular cushion. Adapted from Netter FH. Atlas of Human Anatomy. Basel, Switzerland: Ciba-Geigy Ltd; 1989.92

Embryological Building Blocks of the Formed Heart
A fate map of the formed heart may facilitate an active understanding of septation. Fate maps obtained by following physical markers are only available for avian embryos,3 but as shown above, useful data can also be obtained by following the distribution of phenotypical markers and the analysis of congenital malformations. Figure 4 shows a fate map of the human heart, mapping the structures described in this review.

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