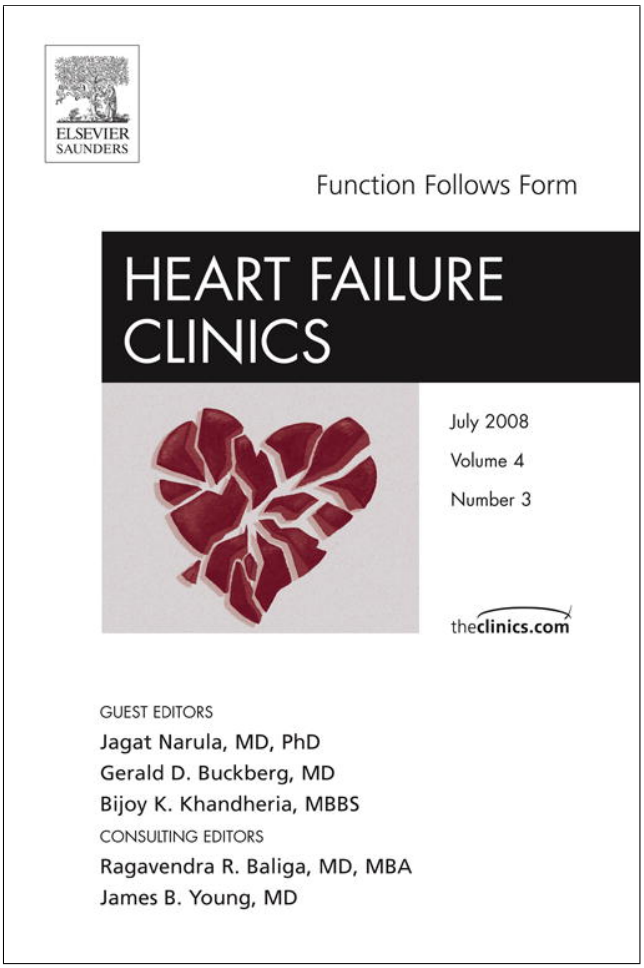

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Embryogenesis of the Heart Muscle

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This article focuses on the development of two distinct components of the myocardium: the so-called "working myocardium," which encompasses the bulk of the cardiac mass, and the specialized network of pacemaking and conduction myocardium, known as the cardiac conduction system. Of course, the heart contains other critical tissues, including serous and fibrous covering (epicardium), fibrous tissue derivatives (valves and cardiac skeleton), blood supply (coronary arteries and cardiac veins), and an endocardial lining. Each of these tissues is important for proper heart function, and the developmental story of each is the subject of much ongoing research. For those interested in an overview of these dynamic subfields and for an introduction to these topics, the reader is referred to a recent dedicated handbook, ML Kirby's *Cardiac development* [1].

Before one can consider the working myocardium and cardiac conduction system populations in detail, the events that lead to the formation of at least the tubular heart from the precardiac mesoderm have to be addressed, and current knowledge of genes involved in myocardial differentiation needs to be summarized.

Early stages of heart formation

The heart takes its origin from paired cardiac mesodermal primordia that fuse in the midline to create a primitive tubular heart [2]. Soon after initiation of a heartbeat, the cardiac tube undergoes a process of looping, which leads to creation of the first grossly visible asymmetry in the embryo. The looped heart then enters a period of chamber formation, with five compartments identifiable by morphologic as well as molecular criteria. Following the blood flow, the first segment is the sinus venosus. The sinus venosus functions as a blood reservoir and pacemaker of the heart, which correlates with its more robust expression of genes necessary for spontaneous action potential generation [3]. Morphologically and histologically, the sinus venosus is characterized by thin walls, small cell size, and scarce intracellular myofibrils. Next come the yet unseparated atrial chambers, with faster impulse propagation, absence of cardiac jelly, and no trabeculae (although they develop the pectinate muscles later on). The third segment is the atrioventricular channel. Atrioventricular channel myocardium is noted for a strongly circular myofiber alignment, as well as a lining of cardiac jelly; it also exhibits a slow conduction velocity. This gives it the role of delay generator for conduction between the atrial and ventricular myocardium (similar to the function of the atrioventricular node in the mature heart). The cardiac jelly is molded into atrioventricular cushions, which participate later on in chamber septation and formation

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of the atrioventricular valves. Next come the ventricles, which are distinguished by development of an extensive trabecular network on the luminal side, fast impulse propagation, and most rapid differentiation of myocytes with respect to their contractility, channels, and energy metabolism.

Most of the remaining discussion will focus on development of the ventricular myocardium, because the ventricles are the main pumping chambers of the heart, and cardiac failure is essentially failure of the ventricles. The last myocardial segment in the tubular heart is the conotruncus, or the outflow tract. Similar to the atrioventricular canal, the conotruncus myocardium also has the characteristic of the earlier primitive tube [4]: that is, slow conduction [5,6] and prevailing circular myocyte alignment. Derived from the secondary heart field, it is a transitory structure that undergoes extensive remodeling to give rise to important structures in the mature heart. The outflow cushions take part in division of the cardiac outlet into an aortic and pulmonary component, and their distal parts form the semilunar valves. The conotruncus myocardium mostly disappears through apoptosis [7], with the exception of the portion forming the pulmonary infundibulum.

Box 1. Genes involved in differentiation of myocardial lineage

Transcription factors (targets)
GATA
Nkx
Myocardin
MEF
Tbx
SRF

Stimulation
Activin
TGFbeta
Wnt (non-canonical)
BMP
FGF
Shh

Inhibition
Wnt (canonical)
Notch
Noggin

Data from Kirby ML. Cardiac development. New York: Oxford University Press; 2007.

Molecular determination of cardiac lineage

The cardiomyocytes differentiate from horseshoe-shaped mesodermal primordia (Box 1). In vitro studies have shown that there are more of these cells capable of forming cardiac myocytes than what actually occurs in vivo [8], pointing to a combination of positive and negative regulators of cardiomyogenic differentiation. A list of currently identified genes participating in commitment to, as well as restriction of this program, derived from a recent reference [1], is provided for overview (see Box 1).

Structure and function of the tubular heart

Although already diversified functionally [9], there is minimal variation in structure along the early cardiac tube (Fig. 1, Video 1). In sections, the tube is composed of a one or two cells-thick myocardial mantle, an acellular cardiac jelly, and the endocardium. The first morphologic sign of regional myocardial diversification can be perceived in more pronounced circumferential arrangement of actin and fibronectin [10] in the inner layer. Regional differences in myofibrillar patterns become more obvious at the looping stage of the rat heart [11,12]. Whether this regional asymmetry is responsible for the process of looping or its direction needs yet to be established.

The tubular heart (see Fig. 1) differs in its mode of contraction from the mature one, or even a preseptation trabeculated heart, which is similar to the adult in, for example, observing

Video 1. Video recording (25 fps) of tubular heart action. Ventral view of a Stage 12 chick embryo in modified New culture. Best viewed as a loop. Available in the online version of this article at: http://www.heartfailure.theclinics.com.

the Frank-Starling law [13,14]. Ventricular contraction in the tube heart is fundamentally asymmetric, a factor not often considered in biomechanical models of the tube heart [15,16]. The fact that the opposite endocardial surfaces contract to fully occlude the cardiac lumen in a contractile wave from the atrioventricular canal to the muscularized outflow tract has implications for determination of cardiac function at these

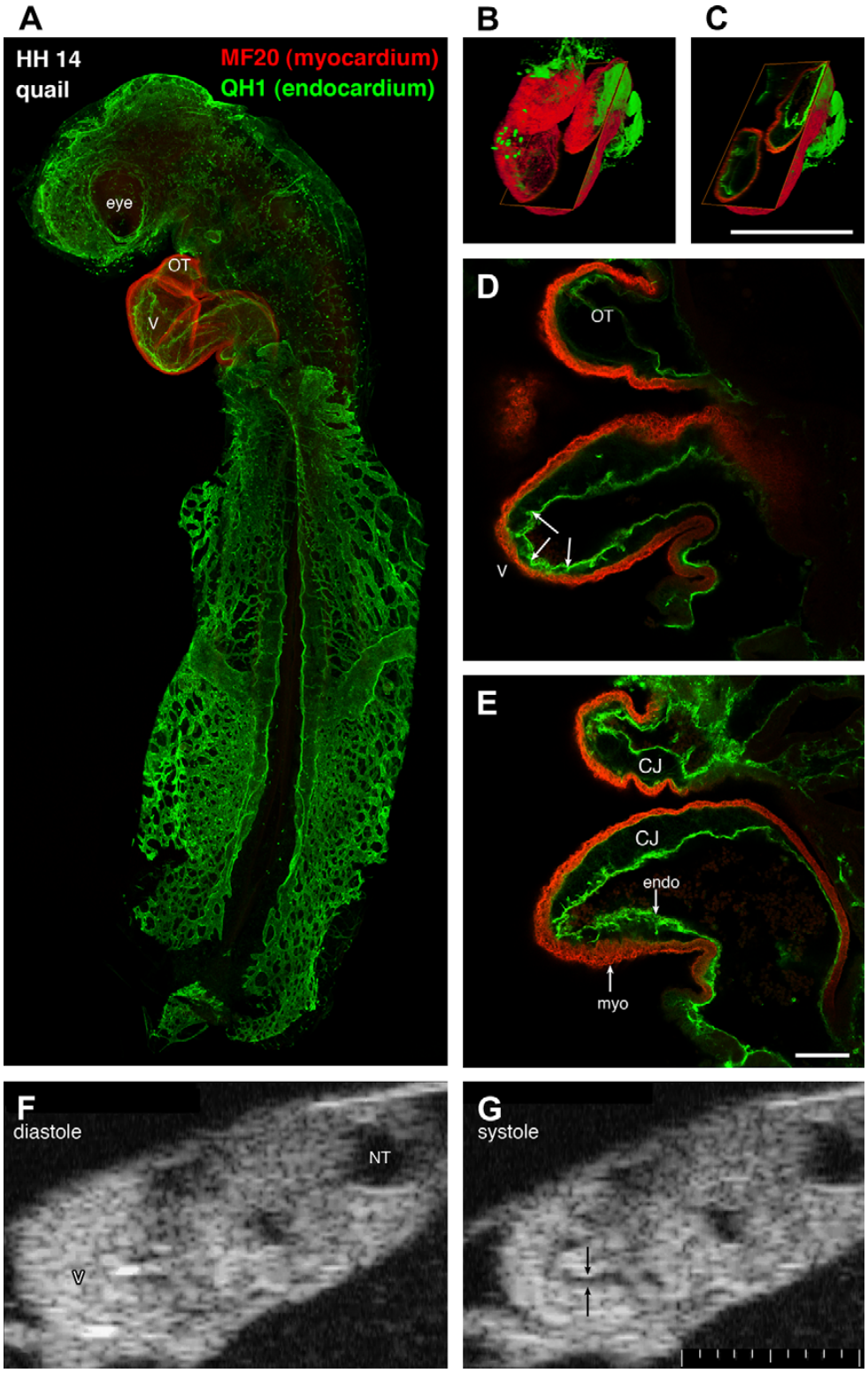

Fig. 1. Structure of a tubular heart. (*A–E*) Whole mount immunostaining for myocardial marker myosin heavy chain (*red*) and endothelial QH1 marker (*green*) shows beautifully the shape of the heart (S-loop). Individual confocal sections (*D, E*) show the diminished amount of cardiac jelly in the ventricular apex, resulting in close contact between these two layers. Endocardial ruffles (*arrowheads*) show the site of prospective trabecular formation. Three-dimensional reconstructions (*B, C*) show the whole heart and an oblique section illustrating the variable extent of the cardiac jelly. Scale bars are 500 μm (*B, C*) and 100 μm (*D, E*). Echocardiographic views (*F, G*) show the heart loop in two different phases of cardiac cycle. Note the echo-free acellular layer of cardiac jelly (*arrows*) along the inner curvature during systole. CJ, cardiac jelly; endo, endocardium; myo, myocardium; NT, neural tube; OT, outflow tract; V, ventricle. Scale bar 100-μm smallest division.

stages. There is no residual nonejected systolic volume under normal conditions until the ventricular cardiac jelly regresses and cardiac ventricular outgrowth (''ballooning'') begins [4]. The ejection phase of contraction in the early tube heart therefore has little in common with ventricular systole in the septating and septated heart with their apex-to-base electromechanical activity. Because there is complete occlusion of the lumen at each point along the early tube heart (see Video 1), there is no back flow, and perhaps no dependence on valves as the whole tube acts as an impedance pump [15,17]. The relevance of measurements of global systolic performance based on calculations of fractional shortening, ejection fraction, and end-systolic volume measurements for measurement of cardiac function during these stages of development is not clear.

Development of myocardial trabeculation

The development of myocardial architecture of the ventricular wall passes through several distinct steps (Fig. 2, Video 2). At first, in the tubular heart, the myocardium has an epithelial character with just two layers of cells. The second step is the formation of sheet-like myocardial protrusions into the lumen, so-called ''trabeculations'' (see Fig. 2). The next step is compaction of the basal portions of these trabeculations, which correlates with the establishment of the coronary vascular system derived from the epicardium. The final stage is the development of multilayered spiral system.

It is possible, only after looping, to discern changes in the luminal appearance of the different components of the tube (see Fig. 1), with trabeculations first becoming evident along the inner

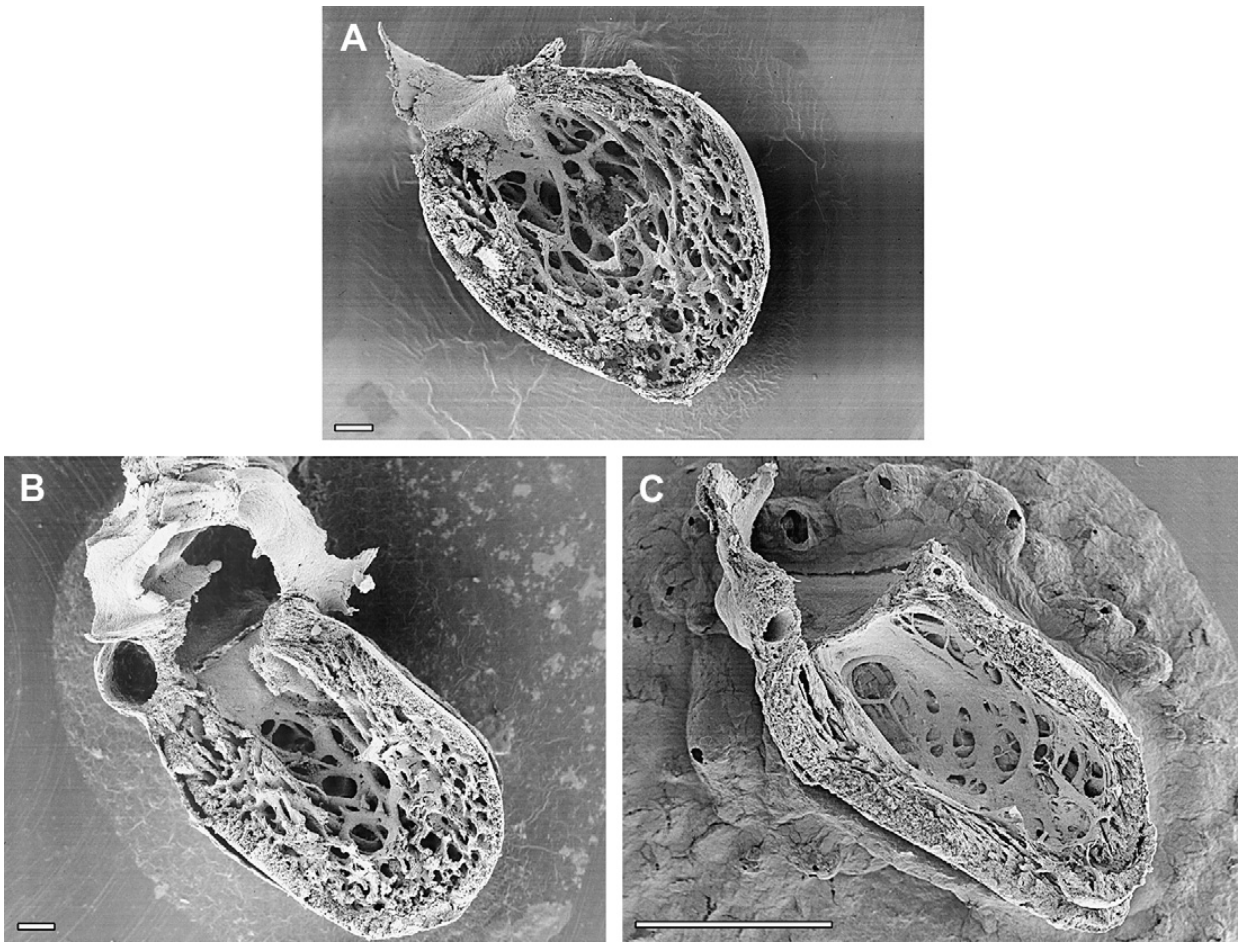

Fig. 2. Time course of ventricular compaction in the human left ventricle. Note increasing proportion and thickness of the outer compact layer. (*A*) Numerous fine trabeculae are present at 6 weeks. (*B*) The trabeculae start to compact at their basal portion, contributing to added thickness of the compact layer at 12 weeks when ventricular septation is completed. (*C*) The compact layer forms the bulk of the myocardial mass after completion of compaction in the early fetal period. Scale bars 100 microns (*A, B*), 1 mm (*C*). (*A and C from* Sedmera D, Pexieder T, Vuillemin M, et al. Developmental patterning of the myocardium. Anat Rec 2000;258:319; with permission.)

Video 2. Ultrasound recording of preseptation trabeculated heart. Four-chamber view of a Stage 24 heart shows the reciprocating contraction of atria (*center*) and septating ventricle (*right*), as well as flowing blood in the branchial arch arteries (*left*) and paired dorsal aortae (*far left, in cross section*). Recorded at 30 fps, smallest division on scale bar 100 μm. Available in the online version of this article at: http://www.heartfailure.theclinics.com.

myocardial layers near the maximum or greater curvature of the looped primitive ventricle [19,20]. This happens in the chick embryo at stage 16/17, in a mouse at 10 days of gestation, a rat at 11 days, and in human beings at the end of the first month of gestation (Carnegie stage 12, the embryo is approximately 4-mm long [21]). The pattern of early trabecular ridges in the ventricular apex runs dorsoventrally (circumferential to the heart tube) and appears similar in different species [18]. At this early stage, the tube itself still contracts in a fashion most consistent with an impedance pump [22]. Maximal stresses and the most highly differentiated cells are concentrated in the myocardial layers adjacent to the lumen, where they may serve to stimulate early trabecular formation [23]. The spacing of such trabecular sheets may depend upon material properties [24,25] of the ventricular wall that result in buckling to relieve tension in response to compression along its longitudinal axis, similar to a long thick carpet pushed from both ends. The early trabeculations effectively increase the surface to volume ratio, enabling the myocardial mass to increase before establishment of a coronary circulation, and may also serve to compartmentalize blood flow as separate ventricular streams before septation [26].

After buckling, the distribution of stresses is no longer uniform, which favors growth of the trabeculations toward the center of the ventricle (see Fig. 2). The trabeculae become transformed into fenestrated trabecular sheets with no free ends; this is conceptually and topologically different from villi in the intestine [27]. Patterns of trabeculations specific for the morphologically left, as opposed to the right, ventricles become apparent at the beginning of ventricular septation [28,29].

The exact patterns of trabeculations in the adult heart show appreciable variability between individuals, and, similar to fingerprints, they seem to be unique for each individual heart. The arrangement of the moderator band, which contains the right bundle branch, and the anatomy of the medial papillary muscle complex of the mitral valve are more uniform, but by no means constant [30].

Atrial myoachitecture

Compared with the ventricle, the atrial myoarchitecture has received much less attention, except as a substrate for propagation of atrial arrhythmias [31,32]. The pectinate muscles appear in the future atrial appendages after the beginning of the atrial septation. A preferential conduction pathway appears during development within the roof of the atria, transmitting the impulse rapidly from the right-sided sinoatrial node to the left atrium. The morphologic substrate of this pathway—the bundle of Bachman—is apparent from embryonic day 6 in the chick onward, and is a prominent ridge of pectinate muscles continuous with the terminal crest [33]. Similar activation patterns are apparent in the embryonic rat and mouse (D. Sedmera, unpublished data, 2003–2005). Further acceleration of impulse propagation (relative to interposed atrial myocardium) is noted along the ridges formed by the forming pectinate muscles, which branch from the terminal crest toward the atrioventricular sulcus. Thus, the pectinate muscles seem to serve a dual role: to strengthen the rather thin atrial wall akin to the struts of an umbrella and act as a morphologic substrate of the preferential conduction pathways, which appear to exist to assure synchronous atrial activation and contraction rather than rapid impulse conduction between the sinoatrial and atrioventricular nodes.

Myocardial (non)compaction

Marked developmental modifications, significant in terms of both structure and function, occur in the compact myocardium (see Fig. 2; Fig. 3). In early embryonic stages in birds and mammals [35], and also in hearts of lower vertebrates (summarized in Ref. [36]), almost the entire thickness of the ventricular wall is made up of a trabecular layer, with little or no coronary vascular supply.

The initial thickening of the compact layer is based on cell proliferation [37]. Subsequent thickening occurs because of compaction of trabeculations, this process coinciding with invasion by the

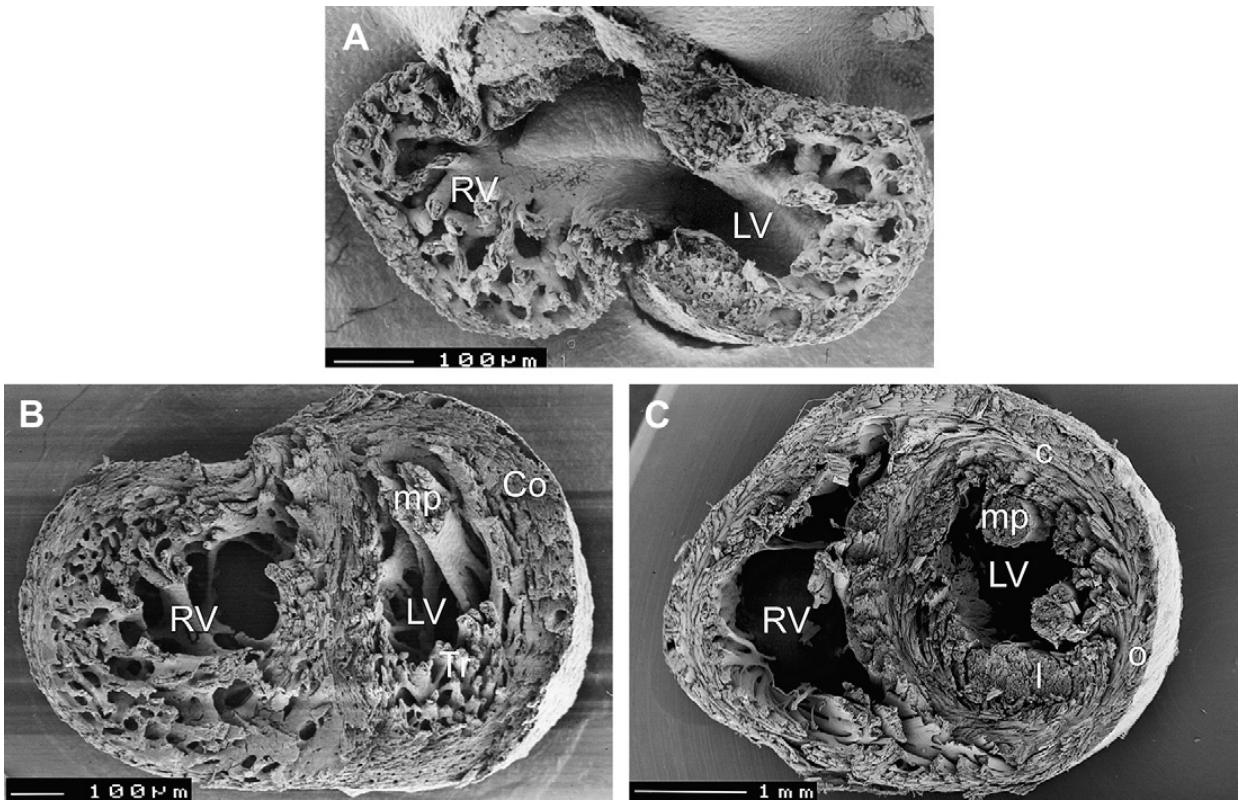

Fig. 3. Development of spiral architecture in the compact layer. (*A*) Pre-compaction stage (E13.5 embryonic mouse heart in transverse section), showing thin, avascular compact layer with circumferential arrangement of myocytes. (*B*) After compaction, vascularized compact layer at E15.5 shows spiral arrangement in both compact myocardium (Co) and trabeculae (Tr). (*C*) In the adult mouse heart, the typical three-layered structure with inner predominantly longitudinal (l), middle circular (c), and outer oblique (o) orientation can be seen in the left ventricle (although recent quantitative studies show that the change in orientation occurs in continuum [34]). Ventricular midportion slabs, viewed from the apex toward the outflow. The first two specimens were prepared by late Dr. Si Minh Pham at University of Lausanne. LV, left ventricle; mp, papillary muscles; RV, right ventricle.

developing coronary vasculature (discussed further in the following section) from the epicardium [38,39]. Compaction is more pronounced in the left ventricle [18], and substantial further growth and compaction occur through the postnatal development [40] with increasing systemic pressure.

The process of trabecular compaction is fairly rapid (in a chick, between embryonic day [E]7 to 10; in a mouse, between E13 and E14; in a human being between 10 and 12 weeks [18]). From E16 in the mouse and the fourth month of gestation in a human being, the compact layer forms the bulk of the ventricular myocardium (see Figs. 2 and 3). Noncompaction of the myocardium presents serious functional consequences for the heart. Several mouse null mutants, such as RXRalpha knockout, present with a complete lack of compaction, which results in lethality around E14 (reviewed in [18]), stressing the importance of the compact myocardium for contractile performance in later fetal stages. In human beings, ventricular noncompaction is usually localized. Although it can occur as an isolated entity, it can be associated with heart failure and sudden cardiac death [41]. Even if only a minor proportion of the ventricular wall is affected, ventricular contractile dynamics may be perturbed [42]. This condition is diagnosed today by ultrasound and is classified as a distinct cardiomyopathy, and several genetic etiologies are recognized [43–46]. There is mounting evidence that the epicardium and the epicardially-derived cells play a crucial role in the development of the compact layer of the ventricular myocardial walls. Not only is the "thin compact myocardium syndrome" observed in several mouse models with perturbed epicardial development, such as in the vascular cell adhesion molecule-1 knockout [47], but experimental studies in avian embryos in which epicardial development is perturbed also show inhibition of ventricular compaction [48]. The exact molecular mechanisms responsible for the regulation of ventricular myocardial

differentiation, growth, and trabecular compaction are currently poorly understood, although retinoid signaling from the epicardium to the myocardium appears to be crucial [49]. More research into this area can bring more clues to the molecular etiology of this condition in human beings.

The progressive thickening of the compact myocardium is accompanied by an improved level of its organization. In 1672 Malpighi had observed that "spiral muscle fibers were successively wrapped" around both ventricles of the chick from the fifth day of incubation onwards. This results in development of a three-layered spiral system of myocardial fibers [50,51]. Development of the spiraling alignment of the fibers (see Fig. 3) likely reflects the twisting pattern of contraction and can be experimentally accelerated by increased workload [52].

Remodeling of the blood supply

Once the size and metabolic activity of an animal exceed the limits of oxygen diffusion, a circulatory system needs to be established. This is true also for the heart, both phylogenetically and ontogenetically. The primitive pulsatile vessels or even sophisticated hearts of more complex invertebrates, such as large marine crustaceans or mollusks, are devoid of coronary vascularization.

If an increase in myocardial mass is necessary, the heart muscle is organized into a spongy network of trabeculae. This arrangement of myocardium is found in larger invertebrates (crabs, oysters), and some lower vertebrates, typically cold-blooded, relatively sedentary animals with low blood pressure, notably certain fish [53,54] and amphibian species, such as Xenopus [36], that have a ventricle devoid of coronary arteries.

The origin of the coronary vessels, including the capillaries, was for a long period controversial. Recent studies using retroviral targeting [55] and chick-quail chimeras [56] clearly showed the extracardiac origin of the angioblasts, which form the primitive vascular bed that will become the coronary vasculature, and in that way supplant older concepts of participation of the trabecular endocardium in the definitive coronary bed, or the sprouting of coronary arteries from the aorta. Indeed, connection to the aorta is the final stage of development (reviewed in [57]). The stimulus for the angioblast invasion is probably local tissue hypoxia [58,59], most pronounced in the outermost subepicardial layers. The mediators are fibroblast and vascular endothelial growth factors. Interestingly, the process of vascularization itself does not seem to be dependent on ventricular loading, as demonstrated by studies of Rongish and colleagues [60]. They used the avascular rat embryonic heart transplanted into anterior eye chamber to study the relationship of the various components of the extracellular matrix to coronary vascularization, and found that angioblast invasion occurred even in the absence of any functional loading.

Unlike most organs in the vertebrate body, the heart has to function continuously from very early stages of its development to support the embryo's increasing demands. Consequently, the coronaryless stages are recapitulated in the ontogenesis of the higher vertebrates, and the lacunar system of the trabeculated heart was recognized more than a century ago [61,62] as a way to increase the surface-to-volume ratio to facilitate the exchange of nutrients and oxygen. The external, coronary blood supply becomes necessary when increasing circulatory demands cannot be met by the trabeculated heart, and increased thickness of the compact layer precludes its adequate nutrition and oxygenation by diffusion from the ventricular lumen.

Development of pacemaking and conduction system

The intrinsic rhythm of the adult heart is determined within the tissues of the cardiac pacemaker: the sinoatrial (SA) node. The SA node is situated at the inflow portion of the heart, at the border of the superior caval vein and the right atrium. Following initiation of a cardiac action potential within the node, the activation wave is propagated through the atrial myocardium, eventually converging into the atrioventricular (AV) node. As its name suggests, the AV node is found at the junction of the atria and ventricles, and functions as a delay generator in the propagation of activation. Following the exit from the AV node, activation rapidly propagates along the His bundle and its branches, finally activating the ventricles via a ramified network of Purkinje fibers. Together, this rapid-conduction system of His-Purkinje tissues forms the last of the main elements of the cardiac conduction system (CCS).

The main components of the CCS show remarkable evolutionary conservation. The development of a mature CCS function during embryogenesis follows significant phases of

cardiac morphogenesis [63]. Initially, the impulse propagates in a slow and apparently isotropic fashion from the sinus venosus, the most caudal portion of the tubular heart, toward the cranially located primitive outflow tract [9]. While the apparent speed of impulse propagation gradually increases (based on total activation time inferred from isochronal epicardial maps), the sequence of ventricular activation as the heart loops still follows the flow of blood. Eventually, the immature base-to-apex sequence of ventricular activation undergoes an apparent reversal, altering to a mature apex-to-base pattern [64–66], which is a hallmark of the His-Purkinje system function.

The cascade of events resulting in the differential gene expression that distinguishes CCS from the working myocardium is becoming unveiled [67,68]. Molecular determinants of the SA node have been recently described [3], and consist of a combination of transcription factors Nkx2.5, Tbx3, and Pitx2c. Nkx2.5 in the atrial myocardium suppresses the expression of pacemaker channel gene Hcn4 and T-box transcription factor Tbx3, restricting their expression domain to the forming of Nkx2.5-negative sinoatrial node and sinus horns and defining a gene expression boundary between the atrium and SA node. Tbx3 in turn suppresses the chamber differentiation program, providing an additional mechanism of reinforcing the nodal identity. Deficiency in Tbx3 results in an expansion of the atrial gene expression program into the nodal area and a partial loss of nodal gene expression, whereas its overexpression reciprocally suppresses the atrial program and induces a pacemaking phenotype [69]. Pitx2c is a laterality determination gene that suppresses the program for SA node formation on the left side, resulting in the normal right-sided position of the node.

Determination of the AV node is linked to formation of the AV canal in the developing heart, or rather, differentiation of the atrial and ventricular chambers from the ''primitive'' myocardium of the cardiac tube [4]. A combination of T-box transcription factors Tbx2 and Tbx5 signaling is involved [70], with Tbx5 being the determinant of the AV canal as well as the ventricular conduction system. Tbx2 is induced by BMP2 expression, and repressed by Hesr1 expressed in the atrial and Hesr2 in the ventricular myocardium [71]. Nkx2.5 signaling is critical not only for the early stages of cardiogenesis [72], but is up-regulated during the later stages of the ventricular conduction system formation, namely differentiation of the Purkinje fibers in the chick [73]. In mammals, it is required for normal formation of the AV node and bundle branches [74].

In the chick, endothelin was identified as a secreted factor capable of turning on CCS markers in embryonic cardiomyocytes in vivo [75], and further studies suggest that it is also involved in turning on the Purkinje fiber-specific program in vivo [76]. There is some evidence that it is also involved in CCS induction in mammals (reviewed in [68]), but further research is needed to confirm this hypothesis.

In summary, myocardium is derived from precardiac mesoderm, and its formation is subject of positive and negative regulation by a number of genes. After formation of a tubular heart with a mode of contraction distinct from subsequent stages, initial increase in ventricular mass during the period of chamber formation is achieved by development of trabeculations, a hallmark of sponge-like hearts. Similar structures (pectinate muscles) develop later on in the atria. Trabecular compaction coincides with deployment of coronary circulation, and results in formation of ventricular chambers with significant, vascularized compact layers and clearly delineated lumen, which are capable of superior performance (pressure generation, ejection fraction) than the avascular, spongy ventricles. The contraction of the developing heart is orchestrated by its pacemaking and conduction system, which develops and changes parallel to heart morphogenesis. While the molecular determination of the sinoatrial node and atrioventricular was recently unraveled, the pathways implicated in induction and patterning of ventricular His-Purkinje system are still subject to research.

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