Function and form in the developing cardiovascular system

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Abstract

Function of the developing heart is dictated by changes in its morphology. For simplicity, we distinguish four stages with different contraction mechanics and conduction parameters. Straight or looped tubular hearts, similar to those of invertebrates such as Drosophila or Ciona, operate as suction pumps and are characterized by a caudally localized pacemaker and slow, peristaltoid conduction and contraction. There is a complete occlusion of the lumen during systole. When the atrial and ventricular chambers appear, the preseptation heart is in many functional aspects similar to the adult heart, but the same function is achieved by different means. There are parallels in design among the hearts of lower vertebrates, such as a spongy ventricle without coronary vasculature and a myocardial atrioventricular canal. Even after septation, considerable maturation of cardiac morphology and function occurs during the foetal and early postnatal period.

Keywords

Myocardium • Conduction system • Embryonic heart • Ontogenesis • Phylogenesis

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1. Introduction

Unlike most other organ systems, function of the cardiovascular system is critical for embryonic survival and, as a result, the heart has to beat in order to support circulation well before its morphogenesis is complete. The function of the heart influences its morphology, and vice versa. The resulting constraints force the hearts into very similar functional designs that are conserved between widely divergent organisms, and make the developmental pathway between the simple cardiac tube and the mature four-chambered organ of homeotherm vertebrates rather tortuous (Figure 1). In this review, I will go through different stages of morphogenesis of the mammalian heart and correlate the structure of the main pumping chamber(s), the ventricle(s), with ventricular function, and give examples of similar functional arrangements from ‘lower’ classes of vertebrates or invertebrates. In parallel, the sequence of activation of different cardiac compartments will be correlated with emerging function and structure of the cardiac conduction system. The main idea is that cardiac design is tightly dictated by demands of life style of the particular organism. Due to evolutionary pressure, this is likely to be a very good fit. As such, we should refrain from calling the designs of ‘lower’ vertebrates2,3 ‘primitive’, especially those of sharks4 should we happen to meet them in their natural habitat.

2. Tubular heart

The first cardiac contractions start at the straight tube stage (Figure 1), approximately E8.5 in the mouse5 and 22 days in humans.6 The first heart beats are irregular and do not propel blood through the circulation,7 but activity quickly becomes coordinated and primitive blood starts to circulate through the embryo, thus enabling its further growth by decreasing diffusion distance for nutrients and oxygen.

Many invertebrates, such as the model organism Drosophila melanogaster, are able to function with this tubular design. In vertebrates, the heart is consistently activated by a pacemaker situated at the inflow part, while in invertebrates with an open circulatory system, the direction of flow can be altered—clearly an advantage allowing sequential perfusion of different parts of the body.8 However, the similarities end at the cellular level, these pulsatile dorsal vessels share properties of skeletal muscle, as is the case of the ‘lymphatic hearts’.9 In the chordate Amphioxus,10 there are multiple pulsatile vessels. Of note, although there is an epicardial layer, the hearts of invertebrates supporting open circulatory systems lack endocardium11,12 (Figure 2).

3. Cardiac looping

The next stage of cardiac morphogenesis is the transition from a straight tube to a looped heart (Figure 1). This process is termed...
Figure 1  Four key stages in cardiac morphogenesis—tube, loop, chamber formation, completion of septation with deployment of coronary circulation. Art work by Ivan Helekal based on chick heart development studies by Manner\textsuperscript{14} and Sedmera et al.\textsuperscript{90,91}

Figure 2  Similarities in design of hearts without coronary circulation. (A) Blue Crab, transverse paraffin section stained with hematoxylin–eosin. (B) Oyster, transverse section through ventricle stained with rhodamine–phalloidine. Scale bars 100 μm. Note that these two invertebrate hearts do not contain endocardium (four times magnified view of trabeculae, direct interface of myocytes with haemolymph indicated by arrowheads in A). (C) Frog, sagittal section. (D) Chick embryo, Stage 29 (embryonic day 6), transverse section through the right ventricle, scanning electron micrographs. Most of the ventricular myocardial mass is contained in the trabeculae (Tr). Outer compact myocardium (Co) is very thin. En indicates endocardium covering the myocytes. Partly based on studies by Sedmera et al.\textsuperscript{47,90}
cardiac looping,13,14 and for some it does not end with formation of the loop, but continues by further ‘twisting’ well through the septation process.15,16

Description of mechanics of the tubular heart,12 initially described as peristaltic,17,18 is now being gradually replaced by a more precise, albeit more complex, suction pump model.19 At this stage, there is complete obstruction of the lumen by apposition of endocardium reinforced by cardiac jelly, as demonstrated using high-resolution ultrasound20 or optical coherence tomography (OCT21,22). Such tubular hearts have 100% ejection fractions, which is never achieved at later stages; this geometry is at the extreme end between cylindrical, optimal for generating pressure, and spherical, optimal for volume displacement.23 In homeotherm embryos, pumping function of the tubular heart is required from this stage on for embryonic survival. Therefore, long-term consequences of perturbations resulting in significant cardiac dysmorphogenesis cannot be analysed in these model species. In contrast, during zebrafish development, the onset of circulation precedes functional requirement by several days, allowing a rare window of opportunity to analyse various mutants with severely dysfunctional or non-functional hearts and dissect the effects of function or blood flow on cardiac morphogenesis.24

The conduction system at that stage has a well-defined pacemaker at the inflow, but the general speed of conduction is slow, correlating with the ‘peristaltoid’ mode of contraction. Gradually, conduction remains slow in the regions of the atrioventricular canal and ventricular outflow, while there is some acceleration in the prospective ventricular region (17,25; reviewed by Gourdie et al.26). For illustration, see the Supplementary material online, Movie S1.

4. Chamber formation

Soon after the beginning of looping, morphological differentiation of myocardium along the cardiac tube becomes apparent.27 This includes disappearance of cardiac jelly in the atria and ventricles, coinciding with the process of development of trabeculae, and, soon afterwards, pectinate muscles in the atria (Figure 3). The morphological differences are accompanied by changes in gene expression patterns.28,29 This process is well conserved in vertebrates, no matter whether there is one or two atria or ventricles. Ventricular trabeculae provide a means of increasing myocardial mass in the absence of coronary circulation30 and are also seen in the hearts of more complex invertebrates (Figure 2). Some of these (mollusks) even have separate atrial and ventricular chambers separated by a valve.12

Recently, a cardiac phenotype very similar to that of ErbB2 null mice lacking ventricular trabeculae31 was described in zebrafish,32 showing conserved mechanism of ventricular trabeculation initiation via endocardium–myocardium neuregulin-Erb signalling in vertebrates.33 In this respect, it is interesting to speculate about the

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Figure 3 Comparative morphology of adult atrial and ventricular chambers in the zebrafish and mouse. Despite considerable evolutionary distance and dramatically different life styles, the main features of chamber myocardial design (ventricles with trabeculae carneae, Tr; atria with pectinate muscles, pm) are conserved. Not visible is the similar ventricular activation pattern from the apex.47,72 In the zebrafish, this is achieved by trabeculae connecting the atrioventricular canal myocardium with ventricular apex (curved arrow), while in the mouse, by His bundle and its branches contained in the interventricular septum. The differences are in higher proportion of trabeculated ventricular myocardium and presence of a single atrial and ventricular chamber in the fish (expertly dissected and imaged by Norman Hu, Utah). Previously published scanning electron micrograph from Sedmera et al.91 and related to Wessels and Sedmera.70
molecular mechanism of trabecular formation in invertebrates (Figure 2) that lack the endocardium. The conduction system starts to show mature characteristics once the chambers are formed, i.e. activation from a well-defined pacemaker, spreading through the atrial tissues using preferential pathways, and atrioventricular delay based upon slow conduction through the myocardium of the atrioventricular canal. The outflow valve function is exercised, rather efficiently, by a combination of close apposition of the outflow tract cushions and contraction of the slowly conducting myocardium of the outflow tract. (Figure 4, Supplementary material online, Movie S2).

At this stage, the embryonic heart is big enough for imaging and acquisition of functional parameters. It was shown that many functional aspects of the trabeculated heart are comparable with the adult heart, and the same parameters can be meaningfully determined after some modification of the equipment. Video recordings (Supplementary material online, Movie S2) of chick hearts, together with direct servo-null pressure measurements and Doppler flow waveforms, have taught us that the trabeculated embryonic heart observes the Frank–Starling relationship, dorsal aortic blood flow scales with embryo weight, and that the increase in heart rate results in decreased cardiac output.

Video recordings in mice followed shortly, and ultrasound studies even allowed longitudinal follow-up of individual embryos and provided functional insights into the demise of some prenatally lethal mouse mutants, such as NFATc1 mutants that lack outflow valves. Likewise, insufficient valve function of the cushions in the outflow tract in ED11.5 mouse embryos leads to diastolic flow reversal and death at ED12.5.

Recently, results from early human embryos have been reported using advances in gynecological ultrasound.

5. Maturation of ventricular impulse conduction

The heart with formed cardiac chambers is from the conduction system point of view directly comparable with the matured one. The pacemaking activity is localized to the sino-atrial node, which starts to acquire its autonomic innervation pattern for neural modulation. The function of delay generation between the activation of atria and ventricles begins to shift from the slowly conducting myocardium of the atrioventricular canal, that is similar to arrangement in the adult hearts of lower vertebrates to the atrioventricular node. The transition from primitive base-to-apex activation of the ventricles to mature apex-to-base sequence correlates with ventricular septation. This is rather logical, as the proximal part of the fast component of the ventricular conduction system, His bundle and bundle branches, is located in the interventricular septum.

However, the actual transition occurs before the completion of septation, and is structurally supported by trabecular sheets connected directly to the atrioventricular canal myocardium. This is similar to situation in the lower vertebrates where apex-to-base activation is achieved by trabeculae connecting the ventricular apex and atrioventricular canal myocardium (Figure 3), which is insulated from the ventricular compact zone by a fibrous wedge of subepicardial tissue. The first precursor of the ventricular conduction system is the primary ring, described first by immunohistochemistry and later shown to mediate early ventricular activation in the mouse. The chick, and rat. Interestingly, the apex to base activation of the ventricle is first heralded by an epicardial breakthrough localized to the right of the interventricular septum, near the apex of the forming right ventricle. This was documented in the mouse, chick, rat, and rabbit and is indicative of earlier right bundle branch functionality. This precocious functionality suggests that geometry of this bundle—narrow with fewer side branches compared with left bundle branch—plays an important role in conduction speed. Earlier functionality of the right bundle is conserved among species despite molecularly more advanced differentiation of the left ventricle evidenced by earlier and higher level expression of connexin40, shown both in the chick and mouse. Examples of our interpretation of ventricular activation patterns, from the least mature to most, are shown in Figure 5. Conservation and potential utility of this arrangement could be explained by the close proximity of the ventricular inflow and outflow at the base
of the heart, which favours beginning of contraction from the opposite end—the apex.

6. Remodelling of atrioventricular myocardium

The myocardium of the atrioventricular canal initially mediates, together with the atrioventricular cushions, valve function in the preseptation heart. In higher vertebrates, atrioventricular canal myocardium gradually regresses, at least in part through apoptosis, and is replaced by fibrous insulating tissue derived from the epicardium. The function of delay generator between the atrium and ventricle is then localized to the atrioventricular node. This structure is prominent and well recognized in mammals, and has recently been characterized at the molecular level as a rather complex structure with several functional subdomains with differential gene expression. It is well recognized that the adult node is derived from a slowly conducting embryonic myocardial segment expressing, among other genes, Tbx2 that is down-regulated in the developing chambers. A molecular model of conduction system differentiation was recently presented by the Amsterdam group. Detailed understanding of the normal development of atrioventricular canal myocardium is essential for our ability to explain the developmental origins of cardiac arrhythmias such as ventricular pre-excitation, characterized by the presence of rapidly conducting anomalous myocardial connections between the atria and ventricles. Abnormal atrioventricular canal remodelling in the quail model of epicardial ablation can lead to persistence of accessory pathways that can lead in turn to ventricular pre-excitation or form a substrate of atrioventricular reentry circuit resulting in reciprocating tachycardia. This situation is observed in the early embryonic mouse heart, where these connections are still normally present and functional.

Problems can arise also from the opposite situation, where conduction from the atrioventricular node is slowed or even completely blocked. The atrioventricular node is one of the targets of the antibodies present in mothers with lupus. These patients stand up to 5% chance of having a child with congenital heart block. Third-degree atrioventricular block results often in profound bradycardia, leading to circulatory failure with accumulation of fluid in foetal tissues and cavities (hydrops) and rapid demise. Fortunately, diagnosis is nowadays possible in utero, so serial monitoring of at-risk pregnancies is possible and treatment can be initiated as soon as the condition develops; however, no firm therapeutic guidelines are currently established.

7. Foetal circulation

While the interest of developmental biologists wanes after completion of septation, in the clinical settings, this is when it just starts, since the earliest stages under 12 weeks are seldom studied apart
from confirming the presence of a beating heart. Many interesting things happen between septation and birth, especially in humans where this period lasts 6 months rather than 6 days for the mouse. One important step in myocardial morphogenesis and maturation is deployment of the coronary supply to the ventricular myocardium, occurring in all higher vertebrates and some (but not all) lower vertebrates. While the evolutionary necessity of ventricular compaction is debated, its importance for homeotherms is clearly shown by lethality of mouse mutants with impaired compaction (reviewed by Wessels and Sedmera) or hypoxic quail embryos with absent coronary arteries and thin compact myocardium. The foetal heart not only grows in size, but further develops spiralling myocardial architecture of the compact layer. This development was shown to depend on haemodynamic loading, being accelerated by increased pressure load and delayed by decreased preload. Abnormal haemodynamics can lead to development of some forms of severe congenital heart disease, such as aortic atresia in hearts that were perfectly normal at the routine scan at 19 weeks of human gestation. From clinical perspective, the period between 20 and 28 weeks of gestation is the time when foetal intervention can be attempted for some severe lesions with predictably devastating consequences such as severe aortic stenosis. Recently, a rare valvar anomaly (mitral fibrous arcade) was linked to volume overload in the recipient twin in the settings of twin–twin transfusion syndrome, confirming the role of haemodynamic loading not only in myocardial changes, but also valve maturation. Impaired performance of malformed hearts also affects the other foetal systems, most critically the central nervous system. It is thus likely that foetal interventions improving cardiac output, such as balloon dilation of the stenotic aortic or pulmonary valve, could have potential benefits exceeding the simple restoration of normal cardiac anatomy and circulation. Several foetal models were developed to test the effects of prenatal interventions or to study the growth response of the foetal heart to haemodynamic challenge. It is important to know the limits of adaptation of the foetal circulatory system, since some procedures that might work well in theory may not be tolerated by the whole organism (e.g. requirement of three-stage palliation of the hypoplastic left heart syndrome ending up in Fontan circulation rather than ‘all at once’ procedure; reviewed by Sedmera et al.). Interventions in prenatal life have the potential to take advantage of the growth capacity of foetal myocardium based upon myocyte proliferation rather than hypertrophy. Such restoration of myocardial mass with appropriate vascularization might also result in superior performance in the long term.

8. Adaptation to postnatal circulation

Changes in circulatory patterns after birth are well known from embryology and obstetrics textbooks. The early postnatal period differs from subsequent growth in childhood, as there is a time window during which cardiomyocytes are still capable of proliferation. In rodents, this privileged period lasts for about 3 days after birth, while in humans, it is believed to last about 6 months, and surgical interventions such as biventricular repair for hypoplastic left heart syndrome are most successful during this window.

The ability of the adult mammalian myocardium to regenerate is limited. Nevertheless, because of the potential significance of regeneration to counter the current human plague of infarction and resulting chronic heart failure in survivors, exploration of regenerative potential is a subject of intensive research. The identification of resident cardiac stem cells generated much hope as well as much controversy, and the current state of the field is nicely summarized by Rosenthal and Harvey. Proliferative activity of adult cardiac myocytes has recently been documented in the normal human heart. However, it appears to be more related to homeostatic renewal than further growth of myocardium or regeneration after ischaemic attack. Myocardial infarction is healed by scar tissue, thus sacrificing long-term output for immediate strength and decreased risk of rupture. A further insight into normal developmental processes is necessary to define optimal therapeutic strategies for regeneration and repair of the diseased adult heart.

9. Concluding remarks

During heart morphogenesis, cardiac performance increases dramatically and a sequence of different functional designs (tubular heart, spongy ventricles), tested successfully during the course of evolution, meld smoothly into each other. Each morphological stage has a developmentally appropriate pacemaking and conduction system. A better understanding of the links between cardiac structure and function is important for devising treatment strategies for the developing heart and new in vivo imaging technologies, such as high-resolution ultrasound and OCT, are changing the way we look at the structure–function relationship in cardiac morphogenesis and dysmorphogenesis.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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