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Developmental Aspects of the Electrophysiology of the Heart: Function Follows Form

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Introduction

The cardiovascular system is the first organ system to form and function in the developing embryo. The function of the system is to continuously pump blood throughout the body for an entire lifetime. The adult heart, as the main pump in this system, performs roughly two thousand million cycles (2.3×10^9) in a typical lifetime. This continuous cycle is necessary to supply the whole body and all of its organs with oxygen and nutrients. Realization of this requires that the heart relaxes so that its chambers, the atria and ventricles, can fill with blood and then contract to propel the blood throughout the body. To achieve this, an intricate and complex organ developed, containing multiple chambers, nodes, valves, and electrical and force-producing components. In contrast, in primitive chordates and early vertebrate embryos the heart merely constitutes a myocardial mantle enfolding a ventral aorta, in which the blood is propelled by peristaltic contractions. The cardiomyocytes of such a primitive heart can be considered as "nodal" cells as they display automaticity and are poorly coupled, resulting in slow propagation of the depolarizing impulse and a matching peristaltic contraction. Eventually, the development of polarity, specifically, dominant pacemaker activity at the intake of the heart, led to the evolution of a one-way pump. Although dominant pacemaker activity implies development of sinus node function, only in mammals does a morphologically distinct node actually develop.[1] The addition of highly localized, fast conducting cardiac chambers to the straight heart tube is an evolutionary novel event, and resulted in the four-chambered hearts of birds and mammals with synchronous contraction for a dual circulation. Interestingly, concomitant with the formation of chambers, an adult type of electrocardiogram (ECG) can already be monitored in the embryo (Figure 2–1).[2] Thus, cardiac design, e.g., the positioning of the atrial and ventricular chambers within the straight heart tube, rather than the invention of nodes, principally explains the coordinated activation of the heart reflected in the ECG. To address the question why some areas of the embryonic heart tube do not participate in the formation of atrial or ventricular working myocardium and mature in a nodal direction, we suggest that the chamber-specific program of gene expression is specifically repressed by T-box factors and by other transcriptional repressors. Consequently, aberrant expression of these factors might be at the basis of ectopic automaticity and congenital malformations of the cardiac conduction system in the formed human heart.

Confusing Terminology

In authoritative work by Davies and colleagues, for which they scrutinized numerous anatomical, developmental, and clinical aspects,[3] the conduction system is defined as the system that initiates and conducts the sinus impulse. In their view it was composed of the sinus node, atrioventricular node, atrioventricular bundle, and the bundle branches and their ramifications. However, the

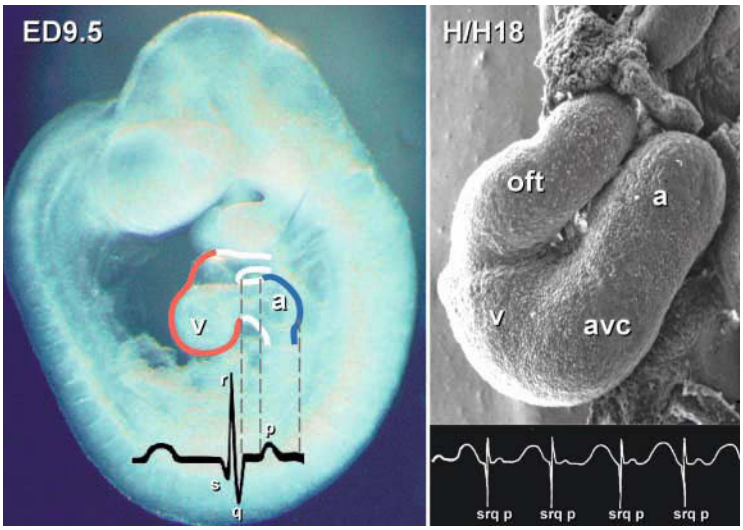

FIGURE 2–1. Concomitant with the formation of chambers (atria, ventricles), an adult type of electrocardiogram (ECG) can already be monitored in the embryo.[2] Scanning electron microscopic photographs of the developing chicken heart with matching electrocardiograms. At H/H 18, locally fast-conducting chamber myocardium has differentiated as reflected in the electrocardiogram. A = atrium; avc = atrioventricular canal; oft = outflow tract; V = ventricle. Note that the ECGs are displayed mirrored to match the position of the chambers in the embryonic heart. The apparent T-wave has not been labeled since it reflects the depolarization of the muscularized outflow tract at this stage rather than the repolarization of the ventricle.

myocardium of the atrial and ventricular chambers were not classified as component parts of the conduction system. This strict dichotomy between conduction system and chamber myocardium was, and still is, the conventional view.

The cardiac electrical impulse of a healthy heart is generated in the sinus node and propagates rapidly through the atrial myocardium toward the atrioventricular node, where the propagation of the depolarizing impulse is delayed. Subsequently, this impulse is then rapidly propagated via the atrioventricular bundle, bundle branches, and their ramifications, finally resulting in the fast depolarization of the ventricular myocardium. The whole sequence of cardiac electrical activity can be registered by ECG, which explicitly includes both the atrial and ventricular chamber myocardium as fast-conducting elements. According to the conventional dichotomy mentioned above, the slow-conducting nodal tissues and the fast-conducting bundle branches belong to the conduction system, while the fast-conducting myocardium of the chambers does not. This division is particularly confusing in the developing heart, in which the separate structures are not yet recognizable, although an adult-like ECG can already be derived from the embryonic heart (Figure 2–1).[2,4]

In the view of the above-mentioned inconsistencies in terminology, it is not surprising that the cardiac conduction system and its development have been surrounded by controversy, and therefore we have tried in this chapter to present in simple terms what we regard as a primer for the design and development of the cardiac conduction system.

Early Peristaltic Hearts

Circulatory systems are composed of pumps and transporting vessels. In practice, nature uses two arrangements to make muscle-pumping devices. One version, also utilized by the intestine, uses peristalsis as a driving force, while the other version, present in adult vertebrates, uses chambers and valves (see the next section). In the peristaltic version, a wave of contractions runs along the muscle mantel enfolding the main blood vessel, and this action pushes ahead the encompassed fluid in either direction. Such a rudimentary system is not particularly efficient, but allows the steady movement of fluids and slurries without interfering or obstructing valves and chambers. During evolution, polarity evolved in this primitive peristaltic chordate heart and this resulted in

dominant pacemaker activity at one end of the cardiac tube, transforming such a heart into a one-way pump. It was demonstrated that retinoic acid is involved in this anteroposterior patterning.[5] All regions of peristaltic hearts possess poorly coupled cells and intrinsic automaticity, by which depolarizing impulses propagate slowly along the tube, resulting in matching peristaltic waves of contraction.[6–10] Such slow contractions do not require well-developed contractile structures as are present in the chamber myocardium of higher vertebrates.

Development of Chambered Hearts

It is important to appreciate that the basic characteristics of muscle cells comprising a peristaltic heart are similar to those comprising the nodes of a chambered heart,[11] as this facilitates the understanding that the design of chambered hearts is derived from the peristaltic heart. Though they share design characteristics, the chambered heart and its associated functional requirements are obviously far more complex than a peristaltic heart. Chambered hearts are the more powerful hearts that can cope with the increasing demands imposed by a growing microcirculatory resistance due to the evolutionary development of liver and kidneys. To achieve this, the atria became the drainage pool of the body to allow efficient filling of the ventricles, while the ventricles themselves became the power pumps. Like peristaltic hearts, chambered hearts are directional because dominant pacemaking activity remains localized at the intake of the heart. A logical further addition to chambered hearts involved one-way valves at both the inflow and the outflow of a chamber. Because of this, with relaxation, a chamber could to be prevented from refilling from the downstream compartment, and this prevented, with contraction, backflow into the preceding compartment. Remarkably, the areas in which these valves evolve display many nodal characteristics, and are the same areas that in the vertebrate embryonic heart will not, or will only later develop into chamber myocardium.[12] Thus, cardiac valves are always found in nodal regions. This holds true for the sinoatrial region, the atrioventricular junctional region, and also the myocardial outflow region of the embryonic heart. Interestingly, the outflow tract myocardium in humans can extend as far downstream as the semilunar valves. Spontaneous activity and even tachycardias originating from this area have been reported,[13] underscoring the notion that this myocardium is persisting embryonic nodal-like tissue.

In a broad view, the vertebrate chambered heart can be considered as a tube with nodal-like tissue, which conducts slowly, in which at the dorsal inflow side atria and at the ventral outflow side ventricles have developed (Figure 2–2). These ventricles contain the working myocardium characterized by rapidly conducting components. The expression of a number of genes that can serve as markers for working myocardium, such as the atrial natriuretic factor (*NppA*) and gap-junctional channels (see below) *Connexin 40* and *Connexin 43*, illustrates this process.[14–17] For example, at mouse embryonic day E8–8.5, the expression of these marker genes for ventricular and atrial chamber myocardium can already be observed at the ventral side of the heart tube.[18] This region will expand ("balloon") to form the embryonic left ventricle at the outer curvature.[14] Somewhat later, right-ventricular and atrial expression of the chamber markers is observed at discrete sites of the outer curvatures, these being the regions that will expand to form the respective chamber compartments. Importantly, the sinus venosus, atrioventricular canal, inner curvatures, and the outflow tract do not express chamber markers and will not expand. These structures retain their original embryonic phenotype, and will give rise to the nodal components of the conduction system, except for the outflow tract. This view is supported by the work of both Thompson *et al.*[19,20] and our own laboratory, which demonstrates that these areas indeed do not participate in the formation of the chambers and display low proliferative activity.[21] Moreover, work by Burch and co-workers strongly suggests that the myocardium of the atrioventricular node and atrioventricular bundle share a developmental origin.[22] Additionally, Gourdie and co-workers showed that slowly proliferating myocardium will mature into the nodal lineage with the use of lineage-tracing experiments and birth-dating studies.[23]

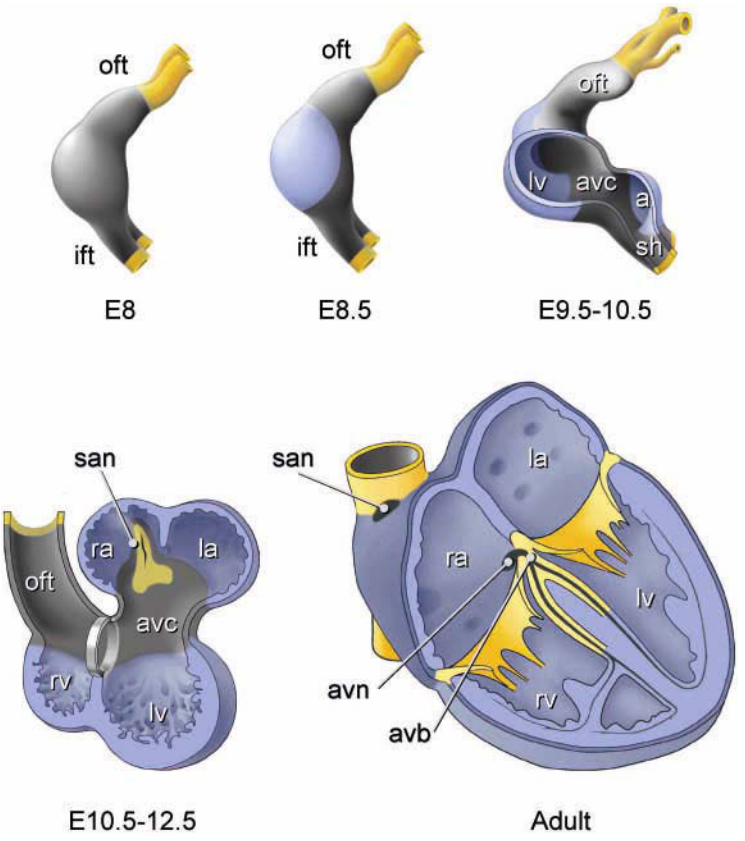

FIGURE 2–2. Schematic overview of heart development in higher vertebrates. Chamber myocardium (blue) expands from the outer curvatures of the primary heart tube, whereas nonchamber myocardium (gray) of the inflow tract (ift), sinus horns (sh), atrioventricular canal (avc), outflow tract (oft), and inner curvatures does not expand. Sinus horn myocardium gives rise to the sinoatrial node (san) and atrioventricular canal myocardium to the atrioventricular node (avn) and atrioventricular junction. The first three panels show a left-lateral view of the heart.

T-Box Transcription Factors Regulate Compartmentalization of the Heart

An important question is why some areas of the (embryonic) heart do not participate in the formation of atrial and ventricular working myocardium and mature in a nodal direction, such as the sinus venosus and the atrioventricular canal. To gain insight into this process we studied the regulation of the *Nppa* gene in more detail. *Nppa* is never expressed in nodal tissues from fish to humans, and in the embryonic heart it marks the developing atrial and ventricular working myocardium.[14] While investigating the mechanism behind the chamber-specific expression of *Nppa*, we established that both a single TBE site (DNA binding/recognition site for T-box transcription factors) and an adjacent NKE site (Nkx2-5 binding element) are present in the *Nppa* promoter and are required for repression of Nppa in the atrioventricular canal[24] and outflow tract.[25] T-box factors are evolutionary highly conserved transcription factors that are important regulators of (cardiac) development. Presently, at least 17 different T-box genes with diverse functions in development and disease are known.[26] In a search for the T-box factors that could act as a repressor for the *Nppa* gene, we observed that *Tbx2* is expressed in inflow, atrioventricular canal, inner curvature, and outflow myocardium. Moreover, expression of *Tbx2* and *Tbx3*, a transcriptional repressor with a similar role, is confined to primary (nonchamber) myocardium, remarkably mutually exclusive to *Nppa*, *Cx40*, *Cx43*, and other chamber-specific genes.[24,27,28] These findings point to a model in which chamber formation (e.g., atria, left and right ventricle) and differentiation are driven by broadly expressed factors, in addition to which a supplementary layer of localized repressors inhibits this process

in regions where chambers do not develop.[18] *Tbx2* gain and loss of function experiments have demonstrated that *Tbx2* is indeed able and required to inhibit chamber formation and expression of chamber marker genes.[27,29] *Tbx3* is expressed in a subdomain of the *Tbx2* domain, and whereas it is able to block chamber formation when expressed ectopically, its deficiency does not lead to obvious defects in atrioventricular canal patterning, indicating functional redundancy with *Tbx2* (our unpublished observations).

But how do *Tbx2* and *Tbx3* exert their functions? Both factors act as repressors of transcription and share DNA binding properties and target genes.[30–34] They effectively compete with *Tbx5*, a transcriptional activator, for TBE binding, and for *Nkx2-5* on NKE binding, thereby repressing chamber-specific genes and chamber differentiation.[18,24,28] Interestingly, although lineage data are lacking, morphological analyses and gene expression studies indicate that the sinoatrial node develops from primary myocardium at the junction between the right sinus horn and the right atrium, whereas the atrioventricular node develops from the atrioventricular canal. The node precursors express both *Tbx2* and *Tbx3*, though during development *Tbx2* becomes downregulated. *Tbx3* expression is maintained specifically in the nodes, thereby providing the only transcription factor found to date to be expressed specifically in the nodes (Figure 2–3).[28,29] As mature nodes display many features that resemble primary myocardium in the embryo, it is attractive to hypothesize that their formation is the result of *Tbx2* and *Tbx3* maintaining the primary phenotype.

Concluding, in a generalizing view it may be envisioned that *Tbx2*, *Tbx3*, and/or other transcriptional repressors suppress the chamber-specific program of gene expression, allowing the regions where these factors are expressed to further mature in the nodal direction. Aberrant expression of such factors might thus be at the basis of ectopic automaticity and congenital malformations of the cardiac conduction system in the formed human heart. Obviously, the spatio-

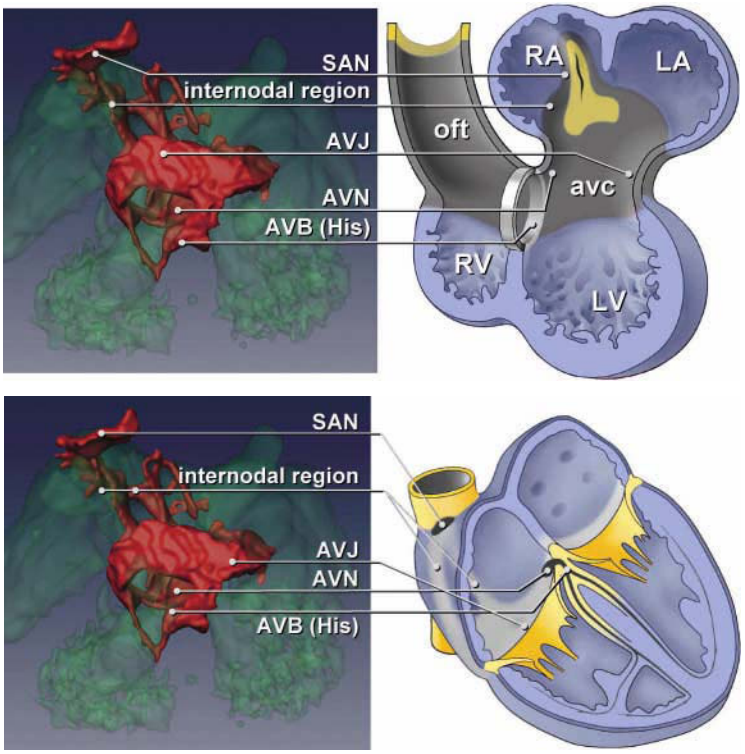

Figure 2–3. (A, B) Tbx3 (red) expression visualized in the heart at ED10.5, marking the sinoatrial node (SAN), internodal region, AV junction (AVJ), AV node (AVM), and the AV bundles (AVB) in respect to the location of the atria, the atrioventricular canal, the ventricles, and the outflow tract on ED10.5 and in adult heart.

temporal regulation of these repressors is the next issue to be resolved.

Tbx5 Segregates the Left and Right Ventricle, Implications for Arrhythmias?

Tbx5 is not only of interest because it is a transcriptional activator involved in chamber formation, as mentioned above, but also because *Tbx5* is expressed in an anteroposterior gradient in the heart tube, which is regulated by retinoic acid.[35] As the left and right ventricles are specified along the anteroposterior axis, it is not surprising that the left ventricle expresses more *Tbx5* than the right ventricle.[36] Moreover, ectopic expression of *Tbx5* in the developing ventricles results in interventricular septal defects and a single ventricle with left ventricular identity.[37] This suggests that *Tbx5* is necessary for left ventricular identity, and provides, in part, the boundary between the left and right ventricle. Actually, mutations in *Tbx5* in humans cause, among other things, septal defects (see below). Consequently, target genes of *Tbx5* are likely differentially regulated between the left and right ventricles. Indeed, one such target gene, a connexin (*Cx40*) (see below), is differentially expressed in the adult heart between the right and left ventricles. Interestingly, recent work has shown that transcription factors can directly regulate the expression of ion channels, since the homeodomain transcription factor *Irx5* was demonstrated to establish the cardiac ventricular repolarization gradient.[38] Seeing that the right ventricle, in particular, is prone to the development of various arrhythmias, such as those seen in Brugada syndrome[39] and arrhythmogenic right ventricular dysplasia (ARVD),[40] and given the fact that *Tbx5* is actively involved in segregating the left and right ventricle, it is tempting to speculate that target genes of *Tbx5* might contribute to or oppose arrhythmias. Recent work has shown that numerous genes are influenced by differences in *Tbx5* dosage, including genes expressed during heart development such as transcriptions factors (*Tbx3*, *Irx2*), cell–cell signaling molecules, and ion channels (*Cx40, KCNA5*),[41] thus indicating that further investigation into *Tbx5* and its downstream genes in relation to arrhythmias and ion channel genes is warranted.

Mutations in Transcription Factors Cause Congenital Heart Defects

As the above mentioned transcription factors are essential for proper cardiac (conduction) system development, it is hardly surprising that mutations in these key genes lead to congenital heart defects. Mutations in *Tbx5* in humans lead to the Holt–Oram syndrome (HOS), which is characterized by anterior preaxial limb and cardiac malformations.[42] All affected individuals exhibit upper limb radial ray malformations that range from subtle carpal bone abnormalities to overt proximal defects such as phocomelia. Many have congenital heart disease such as atrial septal defects (ASD) or muscular ventricular septal defects (VSD), or multiple and complex malformations. It has recently been shown that the skeletal defects seen in HOS patients are likely due to disturbed control of *Tbx5* on the expression of *Sox9* (a transcription factor essential for chondrogenesis and skeleton growth) via control of the expression of connexin 40.[43] This underscores the notion that connexins are involved not only in propagation of cardiac impulses but also in morphogenesis (see below). Mutations in *Tbx3* cause ulnar-mammary syndrome characterized by defects in breast development, apocrine gland, limb and genital formation,[44] and as one study reports, ventricular septal defects and pulmonary stenosis.[45] Moreover, mutations in *Tbx5* interacting partners such as *Nkx2-5* and *Gata4*, like *Tbx5* itself, are known to cause septum defects[46] and, in the case of *Nkx2-5*, atrioventricular conduction defects.[47] Presently no diseases are known to be caused by *Tbx2* mutations. A more common congenital disorder called DiGeorge syndrome is caused by a 1.5–3 megabase genomic deletions of the chromosome 22q11 region, which includes the *Tbx1* gene.[48] Patients with DiGeorge syndrome are characterized by a variety of abnormalities including absence/hypoplasia of the thymus, cleft palate, facial dysmorphism, and cardiovascular anomalies such as aortic arch malformation, outflow tract defects, and VSDs. Recently it was postulated that a synergistic interaction between *Tbx1* and

Nkx2-5 might be responsible for the varying heart malformations of DiGeorge syndrome.[49] As *Tbx1*-deficient mice phenocopy important aspects of DiGeorge syndrome including outflow tract abnormalities,[50] it is believed that *Tbx1* might modulate, in part, the outflow tract defects seen in DiGeorge patients.

Development of the Ventricular Conduction System

The final part of the cardiac conduction system not yet discussed is the ventricular conduction system. The ventricular conduction system mainly develops from the localized trabecular ventricular components (bundle branches and their ramifications) and the primary ring (atrioventricular bundle) as we have reviewed previously,[51] which is in line with the lineage study of Burch and co-workers on the development of the atrioventricular canal, atrioventricular node, and atrioventricular bundle,[22] and with the lineage studies on the development of the bundle branches and their ramifications.[52] Generally, ventricular chamber myocardium develops at the ventral side of the anterior part of the heart tube. An intermediate stage of its development is the so-called trabecular myocardium. While the compact myocardium proliferates exteriorly, the interior trabeculations display low proliferative activity[19,20] and differentiate toward the peripheral ventricular conduction system displaying a high abundance of connexin expression (as mentioned earlier).

Although morphological data are available, few molecular markers exists that specifically delineate the ventricular conduction system,[53] so developmental understanding in this area has lagged behind. However, it was recently observed that in both the embryonic mouse and chick, the ventricular conduction system components are in relative proximity to the cardiac endothelium. Neuregulin-1 is expressed by day 8.5 in the ventricular endocardium, whereas its receptors (ErbB2/ErbB4) are present in the underlying myocardium, making this signaling pathway an excellent candidate for regulation of the induction of the ventricular conduction system. Indeed, mice deficient in neuregulin, ErbB2, or ErbB4 die early with a lack of ventricular trabeculae.[54] Moreover, using a mouse model carrying a marker for the conduction system, Rentschler and colleagues established that exposure to neuregulin resulted in upregulation of a conduction system marker, and presumably the ventricular conduction system, in the developing murine heart,[55] indicating that neuregulin-1 indeed plays a role in the formation of the conduction system, possibly in a paracrine manner.

Sarcoplasmic Reticulum and Heart Development

Having discussed the building plan of vertebrate hearts and their conduction system, it is relevant to look at the developmental aspects of their most prominent features, namely contraction and electrical activity. Regular beating is already observed in the very early period of heart development from embryonic day E9 onward,[56] as mentioned above. This implies that an intracellular system of contraction and cardiac automaticity is already established. Interestingly, the atrioventricular canal and the outflow tract are characterized by slow conduction velocity,[12] a low level of gap junction and connexin expression,[57] and low SR activity.[58] This supports the idea that these flanking segments can function as one-way valves, because they fulfill all the requirements needed for a long contraction duration resulting in a peristaltic contraction. However, the radical change from a peristaltic slowly contracting heart tube to one with fast-contracting chambers necessitates proper control on free intracellular calcium ions. This, in turn, requires the regulated movement of calcium ions across both the sarcolemmal and sarcoplasmic membranes. Clearance of intracellular calcium can be obtained in two ways, either by extrusion of the calcium into the extracellular space by the Na^+–Ca^{2+} exchanger (NCX) or by the sarcoplasmic–endoplasmatic Ca^{2+}-ATPase (SERCA2) into the sarcoplasmic reticulum (SR). SERCA2 itself is regulated by the nonphosphorylated form of another protein, phospholamban.[59]

The calcium handling system was the first ionic system to be studied extensively during heart development, although mostly in the late fetal heart stage (embryonic day 16.5 and later).[59] At this stage most calcium required for contraction

is derived from the calcium influx through the voltage-dependent calcium channels (L-type and T-type). Although a distinct SR is not morphologically observed in fetal myocytes, calcium influxes nevertheless are capable of triggering calcium releases through the calcium release channels (so-called ryanodine receptors, RYRs).[58] It is thought that in contrast to the mature situation, the calcium store for the RYRs in the fetal myocytes are very small organelles that are located far from the L- and T-type channels on the surface membrane. In this way, calcium flowing through the calcium membrane channels diffuses into the intracellular space, where it stimulates the immature SR. The whole chain of calcium-induced calcium release is thus slowed down, and produces the slow kinetics of calcium signals in fetal myocytes.

In contrast, the expression pattern of the various genes involved in calcium handling has been studied more thoroughly throughout embryonic heart development. SERCA2 and PLB can already be observed as early as the cardiac crest stage (embryonic day 7.5 in the mouse), even before myocardial contraction has begun. At this stage, however, there is already polarity in expression, as SERCA2 is more abundant in the anterior region of the cardiac crest and decreases toward the posterior regions, whereas PLB, in contrast, shows complementary distribution.[60] At the stage of the primitive cardiac tube (E8), several additional calcium-related genes start to be expressed. In correspondence with the previous stage, PLB and SERCA2 are still expressed in opposite gradients, e.g., PLB is expressed more strongly in the posterior regions in comparison to the latter. Interestingly, RYR, NCX, and Na^+–K^+-ATPase are distributed homogeneously over the cardiac tube and remain so throughout further embryonic development of the heart.[60] It thus seems that the control of calcium homeostasis is determined solely by the SERCA/PLB system. At the stage of cardiac looping (E8.5), when the first signs of left/right asymmetry and identity are manifested in the embryo, the calcium-related genes maintain their previous patterns of expression. In the final stage (E16.5), termed fetal heart, SERCA2 is expressed more in atrial myocardium than in ventricular myocardium, whereas PLB is once again expressed in an opposite pattern. Additionally, SERCA2 has low expression in the atrioventricular canal (AVC) and outflow tract (OFT). Interestingly, SERCA2 and PLB display a differential expression between the trabeculated and compact layers of the ventricular myocardium. In contrast, the expression of both genes is very weak in different components of the cardiac conduction system, atrioventricular node, and the bundle of His, which is in line with the nodal-like morphological origin of these components, as discussed earlier. The expression of the other components of the calcium metabolism, RYR, NCX, and Na^+–K^+-ATPase, is homogeneously in the different regions of the fetal heart.

Connexins and Heart Development

The propagation of cardiac impulses is mainly determined by the capacity to rapidly carry changes in the membrane potential of cardiomyocytes. This propagation is mediated by gap junctions, which are aggregates of hydrophobic cell–cell channels that allow the intercellular exchange of ions, metabolites, and second messengers of up to 1 kDa in size.[15] The aqueous pores are formed by serially linked hemichannels (connexons) provided by apposing cell membranes. One connexon hemichannel is composed of six transmembrane proteins called connexins (Cx), which are encoded by 21 connexin genes.[61] Although the main purpose of gap junctions in the heart is the conduction of the depolarizing impulse across the myocardium, evidence exists that connexins also play a role in cardiac morphogenesis, as homozygotic Cx43-knockout mice die shortly after birth from a pulmonary outflow tract stenosis due to cardiac malformation.[62] Analogously, in humans, visceral heterotaxia and hypoplastic left heart syndrome have been found to be associated with mutations of the Cx43 gene.[63,64]

Differences in the expression of the connexins are consistent with a functional myocardium (atria and ventricles) model in which the working myocardium has the capacity to transmit the cardiac impulse more quickly than the adjacent myocardium of the inflow tract, atrioventricular canal, and outflow tract. This guarantees synchronized contraction without the need for a specialized system (as discussed earlier). The expression

of the main connexin, Cx43, is detected for the first time in the embryonic heart stage (E10.5). Cx40 has a similar pattern of expression, although more reduced.[60] Cx40 (from ED9.5) and Cx43 mRNA (from 10.5) are detectable in atria and ventricles, but not in their flanking myocardium (inflow tract, AVC, and OFT).[65] Even though Cx40 and Cx43 mRNA eventually become expressed in the inflow tract, they remain undetectable in the sinoatrial node, the AVC (including the atrioventricular node), and the outflow tract.[15] Expression of Cx40 is maximal in the fetal period and declines toward birth. At the stage of the fetal heart (E16.5), Cx43 is restricted to the ventricular myocardium and it is barely detectable in the atrial myocardium in rat, while the mouse atrium expresses much Cx43.[15,60] Analogous to SERCA2 and PLB, Cx43 expression is low in the trabeculated layer and higher in the compact layer.[60] In contrast, Cx40 expression complements Cx43 expression, as Cx40 is expressed mainly in the atrial chambers and shows a transitory differential expression between the right and left ventricles under the influence of Tbx5, as discussed earlier.

It is interesting to note that based on the expression of Cx40 and Cx43 during development, two populations of myocytes can be distinguished, viz. cardiomyocytes that do not express Cx40 and Cx43, and cardiomyocytes that do express both. The first group includes the myocardium of the sinoatrial node, the AVC (including the atrioventricular node), the bundle of His, and the OFT. The second population includes the working myocardium of the atria and the ventricles, and, later in development, the myocardium of the inflow tract (excluding the sinoatrial node), supporting the hypothesis that the structures that do not express Cx40/43 are derived from the embryonic AVC.

Ion Channels and Heart Development

The regulation of the action potential (AP) is determined by a large variety of ion currents. During the depolarization of the myocytes massive amounts of sodium ions are pumped into the cell, whereas the subsequent repolarizations are characterized by a balance of different potassium currents flowing into and out of the myocyte. Many genes are involved in maintaining this dynamic balance, the most prominent being SCN5A, carrying the initial sodium current, and KCNQ1 and KCNH2, carrying the subsequent potassium currents. Several modulator genes, such as KCNE1,2 and SCN1b, also play a role. As much as is known about their function and expression in the adult heart, virtually nothing is known about the different components of the action potential during the various embryonic stages. Attempts to characterize the various individual currents electrophysiologically during development have been hampered by technical difficulties though some studies were published on this topic.[66–68]

In general, the action potentials and resting potentials in cardiomyocytes are altered greatly during development, e.g., both the rate of rise and the overshoot increase along with the duration of the AP. These electrophysiological changes are mainly produced by developmental changes in ion channels, e.g., changes in the amount, the type, and the kinetic properties. The fast sodium current (mainly encoded by SCN5a and SCN1b), which is responsible for the upstroke of the AP, is markedly increased during development. There are few functional sodium channels present at the earliest stage, but the density increases progressively during development. Though the current has a significant sustained component in the earlier stages, this decreases during development, thereby contributing in part to the abbreviation of the AP.[68] The main potassium current in fetal ventricular myocytes is I_{Kr} (mainly encoded by KCNH2 and KCNE2), whereas I_{Ks} (encoded by KCNQ1 and KCNE1) is lacking or very small, though in the early neonate I_{Ks} becomes the dominant repolarizing current. I_f, or the funny current (encoded by the HCN gene family), is the pacemaker current and as such contributes prominently to cardiac rhythm.[69] As it is essential to the function of the sinus node, it is unfortunate that expression studies of HCN during development are scarce. Though recently it was shown that mice lacking HCN4 globally, as well as selectively from cardiomyocytes, die between ED9.5 and 11.5, displaying a strong reduction in I_f and bradycardia.[70] The few studies that investigated I_f electrophysiologically during development did so only in ventricular cells. They show that I_f is prominent at E9.5 in ventricular myocytes and

decreases together with loss of regular spontaneous activity of ventricular cells toward the neonatal stage, which is accompanied by a subtype switch from HCN4 to HCN2.[71,72] So while the I_f current of the sinus node type is present in early embryonic mouse ventricular cells, the ventricle tends to lose pacemaker potency during the second half of embryonic development. Moreover, kinetics were found to be changed, as the threshold voltage to evoke I_f significantly lowers from neonatal myocytes to adult ones.[73] The developmental expression pattern of the HCN4 gene shows that it can already be detected in the cardiac crescent at ED7.5, while at ED8 it is symmetrically located in the caudal portion of the heart tube, the sinus venosus, where pacemaker activity has previously been reported.[74] Further in development HCN4 becomes asymmetrically expressed, occupying the dorsal wall of the right atrium, and will eventually be restricted to the junction of the right atrial appendage and the superior vena cava in concordance with the site at which the SA node is located in the postnatal and adult heart.[75] In the adult heart, HCN4 is mostly expressed in the sinoatrial node, while expression of HCN2 is homogeneously low in the sinoatrial node, AV node, and both the atria and ventricles.[72] The molecular pathways underlying the developmental regulation of cardiac HCN channels are not yet known.

A recent study on expression of these ion channels during various developmental stages demonstrates that SCN5A is distributed homogeneously throughout the embryonal heart (E10.5), whereas the expression of KCNQ1 and KCNH2 appears to be homogeneous only in the embryonal myocardium.[60] In contrast, KCNE1 is expressed in a dorsoventral gradient with a greater expression in the outflow tract region, while KCNE2 is confined to the atrial myocardium. Preliminary evidence seems to indicate that at the fetal heart stage (E16.5), SCN5A is principally expressed in the inflow tract, i.e., the myocardium of the caval veins, whereas ventricular expression is low.[60] However, this contrasts with the high expression of SCN5A in the ventricles of the adult heart,[72] indicating the necessity to investigate this important ion channel in more detail during heart development. In addition, SCN5A is located in the sinus node in the adult stage, as heterozygous knockout mice for SCN5A exhibit impaired SA conduction and frequent sinoatrial conduction block.[76] Currently, no data exist regarding the distribution of SCN1B in the fetal heart or the distribution of these channels in the cardiac conduction system. The expression of KCNQ1 and KCNH2 is again homogeneous in the fetal myocardium. However, while KCNQ1 transcripts have similar levels of expression in the cardiac conduction system and working myocardium, there is an increase in the amount of KCNQ1 protein in the conduction system (AV node, bundle of His, and right and left bundle branches), suggesting a posttranscriptional control mechanism specific to the conduction system.[60] KCNH2 is, however, expressed similarly in mRNA and protein in the myocardium. The modulator genes to these ion channels have a dynamic pattern of expression, where KCNE1 remains limited to the ventricular myocardium, and KCNE2 is confined to the atrial myocardium.

Conclusion

The heart evolved from a myocardial tube in primitive chordates to a four-chambered heart with synchronous contraction and dual circulation in higher vertebrates. Each cardiomyocyte of a primitive heart can be considered as a nodal cell because it displays automaticity and is poorly coupled, which, together with slow propagation, give rise to peristaltic contraction. The introduction of dominant pacemaker activity at the intake of the heart perfected this into a one-way pump. Subsequently, highly localized, fast conducting cardiac chambers were added to this nodal tube, resulting in the four-chambered heart. Interestingly, concomitant with the formation of such chambers, an adult type of electrocardiogram (ECG) can already be monitored in the embryo. Thus, cardiac design, e.g., the positioning of the atrial and ventricular chambers within the nodal tube, principally explains the coordinated activation of the heart reflected in the ECG. A crucial question is why some areas of the embryonic heart tube do not participate in the formation of atrial or ventricular working myocardium and mature in a nodal direction. As a generalized hypothesis we propose that the chamber-specific

program of gene expression is specifically repressed by T-box factors and by the other transcriptional repressors. Consequently, aberrant expression of these factors might be at the basis of ectopic automaticity, malformations of the conduction system, and congenital heart disease in general.

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