Role of Tbx3 in conduction system development
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Chapter

The cardiac pacemaker and conduction system develops from embryonic myocardium that retains its primitive phenotype

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Abstract

Disorders of the cardiac conduction system occur frequently and may cause life-threatening arrhythmias requiring medication or electronic pacemaker implantation. Repair or regeneration of conduction system components is currently not possible due to limited knowledge of the molecular regulation of pacemaker myocardium. Origin and development of the cardiac conduction system have been subject to debate for many decades. This review will discuss recent advances in our understanding of the molecular regulation of the development of the conduction system. We conclude that the components of the cardiac conduction system originate from embryonic myocardium that has maintained essential features of its primitive phenotype while the adjacent myocardium differentiates into working myocardium.
Introduction

In the adult mammalian heart, the pacemaker and conduction system is responsible for the initiation and propagation of the action potential to orchestrate simultaneous contractions of the atria, and after a short pause, the ventricles. The electrical impulse is initiated in the sinus node, spreads rapidly over the atria, is then directed through the atrioventricular (AV) node to the specialized components of the ventricular conduction system that propagate the impulse rapidly to the working myocardium of the ventricles. During transit through the AV node, which is the only myocardial connection between the atria and the ventricles, the electrical impulse is decelerated to ensure sufficient time for the ventricles to fill during contraction of the atria.

In the adult heart, roughly three kinds of cardiomyocytes can be distinguished: 1) pacemaker cardiomyocytes within the sinus node and the AV node, 2) rapidly conducting cardiomyocytes within the ventricular conduction system and 3) working cardiomyocytes within the atrial and ventricular walls. The most important feature of pacemaker cells is automaticity, which is the result of ion currents through multiple ion channels. Most important contributors to automaticity are the pacemaker channel Hcn4, which is highly enriched in the sinus node\(^1\)-\(^3\) and intracellular calcium handling (reviewed in\(^4\)). Furthermore, little intercellular coupling is present between pacemaker cells to allow initiation of the action potential and to delay impulse propagation, which is crucial for the AV node.

The ventricular conduction system consists of the AV (or His) bundle, the bundle branches and the peripheral ventricular conduction system (PVCS), which is also known as the Purkinje fiber network. The main function of cardiomyocytes within the ventricular conduction system is to propagate the electrical impulse rapidly from the AV node to the ventricular working myocardium to orchestrate a synchronous activation of the ventricles from apex to base. This rapid propagation depends on excellent intercellular coupling through gap junction channels that consist mainly of Cx40\(^5\)-\(^7\) and on high levels of the cardiac sodium channel Na\(_v\)1.5\(^8\). Similar to the sinus node and AV node, the ventricular conduction system displays automaticity and can initiate the heart beat, although the intrinsic frequency is low. Working cardiomyocytes are the laborers of the heart through their basic property to contract. The contractile and energy delivery apparatus are very well developed. Furthermore, working cardiomyocytes are well-coupled to establish simultaneous contraction.

The origin of the three different cardiac cell types has been subject to debate for many years. The main issue was whether the cardiac conduction system is derived from the cardiac neural crest or not. Although the cardiac neural crest plays a regulatory role in the development of the conduction system\(^9\)-\(^12\), it does not contribute materially to the conduction system. Convincing evidence that cardiomyocytes within all components of the conduction system are myocardial in origin was provided by the labs of Gourdie and Mikawa. Single myocardial cells in the early heart tube were labeled through...
retroviral infection. At mid fetal stages, clusters of daughter cells were demonstrated in the conduction system and in the adjacent working myocardium,\textsuperscript{13, 14}, indicating that conduction system and working cardiomyocytes are derived from one common myocardial progenitor that is present in the embryonic heart tube.

In the last 20 years, considerable progress has been made to delineate the developmental pathways and molecular cues of embryonic cardiomyocytes while they differentiate into the three myocardial cell types described above. This review will discuss the evidence that supports the hypothesis that the conduction system (excluding the Purkinje fibers) is derived from primitive embryonic myocardium that is inhibited in its differentiation into working myocardium. The embryonic nature of pacemaker and conduction tissue was suspected, but molecular and developmental evidence was lacking. Three examples.

At the end of the 19th century, famous English anatomist and physiologist Walter Gaskell studied the delay in the contraction wave in the AV canal of tortoises. He concluded that this delay was caused by ‘undifferentiated embryonic muscular tissue’ that was ‘characterized by higher automaticity, but lower conductivity’\textsuperscript{15}.

In the 1920’s, drawings of the developing heart by Didusch and Heard (first published in Streeter in 1945\textsuperscript{16} and later reproduced in a book by O’Rahilly\textsuperscript{17}) illustrate the notion that the heart develops from the straight heart tube by local differentiation into working myocardium, while the remainder of the heart tube, more specifically the AV canal and the outflow tract, remain in essence undifferentiated (Fig. 1A).

In the 1960’s (de Haan) and the 1970’s (Viragh and Challice) histology, morphology and development of the cardiac conduction system were studied in great detail\textsuperscript{18-21}. Histological analysis and electron microscopy revealed that cardiomyocytes within the pacemaker and conduction tissues were less well developed than working cardiomyocytes. These pioneering investigators suggested that conduction system cells are perhaps an embryonic type of myocardium.

Two alternative hypotheses have been postulated for cardiac conduction system formation. These are the ‘multiple ring model’ and the ‘recruitment model’. The multiple ring model hypothesizes that rings of conduction system tissue are present in the tubular heart prior to chamber formation (reviewed in\textsuperscript{22, 23}). When the chambers develop, the flanking segments impose as rings, because they remain small relative to the expanding chambers. The observation of relative constrictions in the inflow, the AV canal, the interventricular region and the outflow are at the basis of this model. It is, however, unlikely that these ‘rings’ are already present in the heart tube prior to chamber formation. To date, no evidence in terms of expression patterns has been presented to validate the notion that the rings are already present in the embryonic heart tube. As will be explained later, recent lineage experiments provide further evidence that existence of conduction system ‘rings’ within the proposed locations in the embryonic heart tube is unlikely.
The conduction system develops from embryonic myocardium.

The recruitment model states that the components of the conduction system form by inductive recruitment of multipotent cardiomyocytes to an initial conduction system framework\textsuperscript{24,25}. This model was proposed for the development of the Purkinje fiber network and later extended to other conduction system components\textsuperscript{13,14}. In our view, the experimental data could also be interpreted as if precursor cells differentiate into either working myocardium or conduction system myocardium that subsequently further proliferates. This specification model, however, was considered unlikely, because earlier experiments revealed withdrawal of conductive cells from proliferation\textsuperscript{26,27}. Therefore, recruitment was considered to be the most likely option. More recent data, however, indicate that rates of proliferation within the developing conduction system are more than sufficient to explain growth of these components\textsuperscript{28}.

Significant progress has been made in the last decades in our understanding of the molecular regulation of conduction system development. Some of these new insights will be discussed in this review.

**Figure 1** A, Reconstruction of the lumen of a developing human heart by Osborne Heard, drawing by James F. Didusch. Note that the ventricular chambers are connected via the primary heart tube. It further illustrates that the ventricles do not develop from a common ventricle and subsequently separate through the process of septation, as is the case in atrial development. B, Schematic overview of heart development in higher vertebrates. The early heart tube has a primitive phenotype (light purple). Chamber myocardium (grey) expands from the outer curvatures, whereas non-chamber myocardium (dark purple) of the inflow tract (ift), sinus horns (sh), atrioventricular canal (avc), outflow tract (oft), and inner curvatures does not expand. First 3 panels show left-lateral views. **Abbreviations:** A-V J’CT indicates atrioventricular canal (junction); TA, truncus arteriosus; ev, embryonic ventricle; (r/l) a, (right/left) atrium; r/l v, right/left ventricle; scv, superior caval vein; r/lbb, right/left bundle branch; pvc, peripheral ventricular conduction system.
Conduction system myocardium shares key features with embryonic myocardium

From early stages of development onwards, conduction system cells within the sinus node, AV node, AV bundle and bundle branches share characteristic features and are easily distinguishable from working cardiomyocytes. They are glycogen rich and contain less T-tubules, fewer mitochondria, and their sarcomeric apparatus and sarcoplasmic reticulum are less-well developed, resulting in poor contractility and a ‘pale’ appearance. These characteristics also apply to embryonic cardiomyocytes within the early heart tube. Moreover, embryonic cardiomyocytes also share functional characteristics with pacemaker myocardium. They display automaticity and propagate the electrical impulse slowly, which results in a peristaltic contraction wave (Table 1).

Table 1

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This table compares key characteristics of embryonic (primary) myocardium with pacemaker myocardium and working myocardium. SR indicates sarcoplasmic reticulum. * indicates that conduction velocity is high in the ventricular conduction system.

Local differentiation and rapid proliferation of the future cardiac chambers

During the process of looping, specific regions within the heart tube start to proliferate rapidly and initiate the working myocardial gene program. Among others, this typically includes up regulation of genes for fast-conducting gap junction channels, of genes that encode sarcomeric components, of genes associated with mitochondrial function for increased energy supply and of genes responsible for the rapid upstroke of the action potential. The two chamber-forming regions are located at the outer curvatures and will become the ventricles and the atria. One of the earliest markers of the developing chambers is Nppa (Anf). The regions in-between these highly proliferative, expanding cardiac chambers retain their ‘primary heart tube’ characteristics (Fig. 1B).

Intriguingly, the simple configuration of alternating fast-conducting chamber and slow-conducting primitive myocardium establishes cardiac function that resembles the function of the mature heart. The electrical impulse is initiated in the leading pacemaker site in the sinus venosus, propagated rapidly through the differentiated myocardial cells
of the atria, after which the atria contract synchronously to pump the blood through the AV canal into the embryonic ventricle. Due to slow conduction in the AV canal, the electrical impulse is delayed to allow complete filling of the ventricle. During subsequent activation and contraction of the ventricle, the AV canal myocardium remains contracted as a result of slow relaxation, thus functioning as a sphincter valve. After ventricular contraction, the outflow tract contracts and relaxes slowly, preventing back flow of blood into the ventricle.

The regions of the embryonic heart tube that have not differentiated into working myocardium, the sinus venosus and AV canal, correspond to regions in which the developing conduction system components are localized. These regions also function as their mature counterparts. It is therefore attractive to hypothesize that the conduction system components develop within these regions of primitive myocardium.

Markers of the cardiac conduction system

Genes or transgenes that specifically mark all the components of the conduction system have not been reported so far, although separate components of the conduction system can be defined molecularly. Several conduction system-specific reporters have been re-evaluated and none of them appeared to be specific for the entire conduction system. The pacemaker channel \( Hcn4 \) is expressed in all components of the conduction system, but low levels of \( Hcn4 \) expression within the working myocardium have been reported. In the adult heart all conduction system components except the PVCS, are marked by the T-box transcription factor \( Tbx3 \). Throughout cardiac development, \( Tbx3 \) is expressed in a continuous myocardial domain that extends from the presumptive sinus node to the future AV bundle domain. In the developing mouse heart, expression of \( Tbx3 \) is present in the looping heart tube from embryonic day (E) 8.5 onwards. \( Tbx2 \), which is a family member of \( Tbx3 \), is also expressed in primitive myocardium. \( Tbx2 \) is expressed in the inflow tract, which is fated to become the embryonic AV canal. After looping, \( Tbx2 \) expression is confined to the AV canal, overlapping with the expression of \( Tbx3 \). After E9, \( Tbx2 \) is also expressed in the outflow tract. Detailed examination revealed that the expression pattern of working myocardial genes in the adjacent atrial and ventricular chambers is strictly complementary to the expression domain of \( Tbx2 \) and \( Tbx3 \), suggesting that these transcription factors play a role in the regulation of the working myocardial gene program (Fig. 2).

\( Tbx2 \) and \( Tbx3 \) are repressors of working myocardial genes

In vitro and in vivo experiments have demonstrated that both \( Tbx2 \) and \( Tbx3 \) are direct repressor of working myocardial genes \( Nppa \) and \( Cx40 \). These findings suggest a mechanism in which these transcriptional repressors inhibit differentiation into working myocardium in the myocardial regions in which they are expressed. This mechanism
Figure 2 At several stages of development and in different conduction system components the expression of Tbx3 is strictly complementary to Tbx3 target genes. In situ hybridization serial sections of a mouse heart. A, Cx40 is expressed in the developing chambers, whereas Tbx3 demarcates the AV canal (avc). B, Cx43 is expressed in the working myocardium of the interventricular septum (ivs), whereas Tbx3 is expressed in a complementary manner in the crest of the septum, demarcating the developing AV bundle (avb) and bundle branches (bb). C, Nppa is expressed in the working myocardium of the right atrium (ra), strictly complementary to the expression of Tbx3 within the sinus node (san).

Abbreviations: ift indicates inflow tract; oft, outflow tract; a, atrium; lv, left ventricle; lbb, left bundle branch; rsh, right sinus horn.
The conduction system develops from embryonic myocardium was further investigated in transgenic mice and embryos that expressed either too much or too little of the gene of interest.

Due to functional redundancy, the effect of loss of function of Tbx2 or Tbx3 is only visible in those compartments of the conduction system that express either Tbx2 or Tbx3. For Tbx3, these compartments are the sinus node and the AV bundle. In the absence of Tbx3, working myocardial genes were expressed within the sinus node domain, indicating that Tbx3 is necessary for repression of working myocardial genes in Figure 3 Ectopic expression of ventricular markers in the conduction system. Section in situ hybridizations of wild-type and Tbx3 mutant mouse hearts. Probes and genotypes are indicated in the panels. A through D, ectopic expression of Cx40 in the Tbx3 (or Cre) positive sinus node domain (black arrowheads). E through G, ectopic expression of Cx43 in the Tbx3 (or Cre) positive AV bundle region (black arrowheads).

Abbreviations: rscv indicates right superior caval vein. For other abbreviations see previous figures.
the sinus node domain\textsuperscript{42} (Fig. 3). The fact that the sinus node is formed and does express \textit{Hcn4}, shows that \textit{Tbx3} is not necessary for the early formation of the sinus node.

Likewise, the developing AV bundle adopts a working myocardial phenotype in \textit{Tbx3} null embryos\textsuperscript{43}, indicating that \textit{Tbx3} represses working myocardial differentiation in the AV bundle domain (Fig. 3). The development of the AV node appeared to be normal in \textit{Tbx2} or \textit{Tbx3} mutant embryos. However, detailed examination of the AV canal of \textit{Tbx2} null embryos, revealed that working myocardial genes were ectopically expressed in the absence of \textit{Tbx2} at the left side of the AV canal\textsuperscript{28}. At the left side of the AV canal expression of \textit{Tbx3} is nearly absent, indicating that \textit{Tbx2} is required for the repression of working myocardial genes and confirming the idea of redundant effects of \textit{Tbx2} and \textit{Tbx3} in the remainder of the AV canal, including the future AV node.

Next, transgenic mice were investigated that ectopically expressed \textit{Tbx3} in atrial working myocardium from mid-gestation onwards. These experiments revealed that \textit{Tbx3} is sufficient to repress working myocardial genes and to induce the expression of pacemaker genes within its expression domain, although these cells had already differentiated into working myocardium (Fig. 4). Moreover, functional pacemaker sites were formed in the atrial myocardium. Isolated left atrium preparations were contracting spontaneously, while this was never observed in controls. These experiments confirm that \textit{Tbx3} is a key regulator in the development of pacemaker myocardium\textsuperscript{42}.

**Gene expression profiling of the developing AV node**

To evaluate the relationship between the AV canal and the AV node, gene expression profiles of E10.5 AV canals were compared with E17.5 AV nodes and both were compared with age-matched working myocardial cells by microarray analysis. At E10.5 ~14,000 transcripts were detected in the AV canal and the same number was detected in the working myocardium. At E17.5 these numbers had increased to ~16,000\textsuperscript{31}.

To evaluate the difference between the AV canal and working myocardium, the number of differentially expressed transcripts was calculated. This calculation revealed that ~2000 transcripts were differentially expressed between the AV canal and working myocardium at E10.5. At E17.5 the number of differentially expressed transcripts increased to ~6500, indicating substantial divergence of the AV canal/AV node and the working myocardium. At E17.5 many more transcripts were found to be differentially expressed than at E10.5. This is due to general myocardial differentiation (+5000 transcripts) and specific working myocardial / nodal maturation (+2000 and +4000, respectively). Most transcripts (75\%) that are differentially expressed in the E10.5 AV canal are also differentially expressed in the E17.5 AV node. Together, these data indicate that the AV node differentiates considerably during development but that the late fetal AV node largely maintains the E10.5 AV canal program that differs considerably from the working myocardium.
Contributions of working myocardial cells to the conduction system are unlikely

Together, the above-mentioned data suggest a mechanism in which specific regions within the embryonic heart tube differentiate into working myocardium, because genes that suppress this specification step are not expressed in these regions. Questions that remain to be answered are: 1) Do cells that loose the expression of Tbx2/Tbx3 later in development differentiate into working myocardium and 2) is this process reversible? In other words, can working myocardial cells differentiate into primitive or conduction system myocardium?

Lineage analysis was performed by crossing transgenic mice that express Cre under control of the Tbx2 locus with a reporter line, thereby identifying the fate of cells that once expressed Tbx2. This analysis revealed that a large proportion of the left ventricle once expressed Tbx228, indicating that a significant number of AV canal cardiomyocytes can and will differentiate into working myocardial cells. This suggests a continuous process of Tbx2-expressing primitive AV canal cardiomyocytes differentiating into working cardiomyocytes upon loss of Tbx2/Tbx3 expression.

There is functional, molecular and morphological evidence that indicates a direct relation between primitive myocardium and the development of the conduction system. But can we reject the hypothesis that working myocardial cells form or contribute to the conduction system? Strictly, we can not. The definite lineage analysis that delineates the fate of working myocardial cells has not been performed or published. In our lab, experiments were performed with a small Nppa promoter fragment driving Cre. By crossing these mice with the R26R lacZ reporter line, all working cardiomyocytes are permanently marked. Nppa is one of the earliest markers of working myocardial differentiation. Because the recombination pattern in the atria was mosaic, several lines were examined. No recombined cells were detected in the AV node area, indicating that there is no contribution of working myocardial cells to the AV node (42, unpublished data 2009).

Conduction system components are connected to one another from the onset

Although the Tbx2 and/or Tbx3 expressing regions of primitive myocardium proliferate much slower than the atria and the ventricles, a continuum of primitive myocardium remains that runs from the sinus venosus, through the future terminal crest towards the AV canal and via the inner curvature to the interventricular ring and the outflow tract myocardium. This continuum of primitive myocardium can be traced in the heart at any stage of development. The presence of this continuum also implies that conduction system components do not have to connect during development, the connection is there from the onset.
In the adult heart, most of the primitive tissues have disappeared, either by differentiation into working myocardium or by apoptosis. Remnants of primitive myocardium, however, can persist. In AV junctional tissues of healthy dogs and pigs, cells were found that resembled nodal cells in their cellular electrophysiology. These cells were located in close proximity to the base of both the mitral and tricuspid valves. Most likely, these cells are remnants of AV canal myocardium. Clinically, arrhythmias originating from the mitral and tricuspid annulus region have been reported.

Figure 4 Ectopic expression of TBX3 in atrial cardiomyocytes results in repression of Cx40 and in ectopic activation of pacemaker channel Hcn4. In situ hybridization on serial sections of prenatal (E17.5) Cre3 (control) and Cre3 crossed with CAT-TBX3 mouse hearts, showing ectopic TBX3 expression within the atrium (B). Atrial expression of TBX3 results in induction of Hcn4 (D) and down-regulation of Cx40 (F).
The conduction system develops from embryonic myocardium

Elongation of the heart tube by addition of cells instead of proliferation

Until a decade ago, the straight heart tube was thought to contain all the precursors of the adult heart. This view implies that there is rapid proliferation within the heart tube. Elegant tracing experiments and proliferation rate analyses have revealed that the early heart tube only represents the majority of the left ventricle and the AV canal. The remainder of the heart is formed by cells that are added to the heart tube later. A progenitor pool located at the venous pole of the heart tube proliferates rapidly and contributes cells to both the venous and arterial pole of the heart. In the process of differentiation into cardiomyocytes these cells decrease their proliferation rate drastically. What are the implications for earlier observations and conclusions?

The most important implication is that causes of abnormal development of the venous pole, the atria, the right ventricle and the outflow tract do not lie within the linear heart tube but in the precursor pool that is added later. For the development of the conduction system it is clearly impossible that all the conduction system components are present in the early heart tube, which dismisses the ring model. Interpretation of expression data during development should also be re-evaluated, as cells in certain cardiac components do not stay in this functional component, but contribute to other cardiac component later in development. A nice example is the reinterpretation of the reported expression pattern of Tbx2 in the heart. In the straight heart tube, Tbx2 expression was observed in the proximal half of the tube, which is described as the ‘inflow and the future atria’. During the process of looping, the expression ‘disappears in the atria and expression of Tbx2 is only observed in the AV canal’ . As we know now, the cells within the embryonic inflow and atria are not the cells of the adult inflow and atria. These cells and their progeny will form the AV canal and the left ventricle. The expression of Tbx2 did not disappear, but these Tbx2-expressing cells got a new position, the AV canal, due to addition of new cardiac cells at the inflow of the heart.

We have mainly focused on the primitive myocardium of the AV canal developing into the AV node. How does the above apply to the other components of the conduction system?

Sinus node

The sinus node develops within the sinus venosus myocardium. The sinus venosus myocardium differentiates from a separate precursor pool, which is added to the heart tube at E9-9.5. Initially, the entire sinus venosus exhibits a ‘primitive’ phenotype with low expression of working myocardial genes and high expression of Hcn4 and therefore the entire sinus venosus displays pacemaker activity. At approximately E10, the transcriptional repressor Tbx3 is induced in a sub domain of the sinus venosus, where it defines the area of the sinus node primordium. After establishment of the sinus node domain, the remainder of the sinus venosus induces working myocardial genes, whereas the expression of Hcn4 is suppressed. In the sinus node primordium, this
induction process is prevented by Tbx3 and the expression of Hcn4 is maintained, which provides a likely mechanism by which the sinus node becomes the leading pacemaker site.

AV bundle

The AV bundle is the most rapidly conducting component of the cardiac conduction system. Essential for this fast conduction is Cx40, which is abundantly expressed in the AV bundle, bundle branches and Purkinje network. Cx40 serves as an excellent marker to distinguish the fast-conducting components of the ventricular conduction system from the AV node and the working myocardium. In the embryonic heart, the developing AV bundle does not express Cx40, nor is the ventricular conduction system required for propagation of the cardiac impulse from the AV canal to the ventricle. Instead, the ventricle is activated directly from the dorsal AV canal through rapidly propagating Cx40 and Cx43 expressing trabecular myocardium.

The AV bundle is specified by a network of transcription factors including Tbx5, Nkx2.5, Id2 and Tbx3, of which Id2 and Tbx3 appear to be the executors. Intriguingly, the expression level of Id2 is unchanged in Tbx3 knockout embryos. Similar to the development of the sinus node and AV node, Tbx3 represses the differentiation into working myocardium within the developing AV bundle domain, where it is expressed from the looping heart tube stage onwards. These findings suggest a mechanism that induces expression of Cx40 in the presence of Tbx3 in late fetal stages, when the mammalian embryo depends on a fast conducting ventricular conduction system. This mechanism could involve a balance shift in the interactions of several transcription factors.

The peripheral ventricular conduction system

The PVCS consists of a network of thin myocardial fibers located directly beneath the endocardium. The PVCS cells express Nppa, Cx43 and Cx40 and conduct the electrical impulse rapidly to the ventricular myocytes. The PVCS develops within the trabecular myocardium of the embryonic ventricle. Therefore, in contrast to the other components of the conduction system, the PVCS is not directly derived from embryonic myocardium, but from working cardiomyocytes that differentiated early in development. Furthermore, the PVCS does not express transcriptional repressors such as Tbx2 and Tbx3, confirming a different developmental pathway that will not be further discussed in this review (for recent review see).
Final remarks

The formation of the components of the pacemaker and conduction system is summarized in figure 5. Most cardiomyocytes are derived from mesodermal cardiac progenitor cells that express cardiac transcription factor Nkx2.5. These cells give rise to all components of the heart except the sinus venosus derived structures. The sinus venosus is formed by cells that are derived from a progenitor pool that expresses Tbx18, but not Nkx2.5. The sinus venosus will give rise to the sinus node and the sinus horns, which will form the sinus venarum and the myocardium around the caval veins and the coronary sinus.

The Nkx2.5+ myocardium of the embryonic heart tube can be divided into two populations. One population that has initiated a chamber myocardium gene expression program and one population that retains the phenotype of primitive myocardium due to expression of transcriptional repressors (Tbx2, Tbx3 and Id2). However, cells within the primitive myocardium continuously lose the expression of repressors and initiate a chamber myocardium gene program, thereby joining the working myocardium. While it is not known whether this process is reversible, it is unlikely that differentiated working cardiomyocytes can become primitive or conduction system cardiomyocytes again.

After differentiation into chamber myocardium cells will further mature into atrial working myocardium in the atria and into compact myocardium and trabecular myocardium in the ventricles. The trabecular layer is derived from chamber myocardium and will give rise to the PVCS. Although the sinus node, AV node, AV bundle and bundle branches are derived from primitive myocardium, these conduction system components mature significantly. As has been shown by gene expression profiling for the developing AV node, most genes differentially expressed at E10.5 are also differentially expressed at E17.5, suggesting preservation of the phenotype, but many additional genes become differentially expressed, indicating significant maturation.

On the other hand, it is important to realize that a significant proportion of primitive myocardium present in the embryonic and fetal heart, as has been shown for the AV canal, does not contribute to the conduction system, but differentiates into working myocardium. We speculate that these cells initiate the working myocardium gene expression program, due to loss of the expression of transcriptional repressors. The mechanism responsible for this loss of expression, however, remains to be elucidated.

Acknowledgements

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Figure 5 Model for cardiomyocyte differentiation into all myocardial components of the heart. The red arrow between primary and chamber myocardium indicates continuous differentiation of primary cardiomyocytes into chamber myocardium. Abbreviations: A/V WM indicates atrial/ventricular working myocardium. For other abbreviations see previous figures.
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