The conduction system of the heart initiates and coordinates the electric signal that causes the rhythmic and synchronized contractions of the atria and ventricles. In higher vertebrates, this system comprises the sinuatrial (SAN) and atrioventricular nodes (AVN) and the “wiring” of the ventricles. The latter comprises the atrioventricular bundle (AVB), the left and right bundle branches (BBs), and the peripheral ventricular conduction system (PVCS). Failure in the function of the nodes or bundles leads to arrhythmias, requiring electronic pacemaker implantation. On the other side, ectopic pacemaker or conductive activity may cause arrhythmias requiring intervention. Recent progress in molecular-genetic and developmental biology has lead to novel insights into the processes underlying the formation of the conduction system. These insights provide a framework that helps to understand pathologies of the conduction system. In this review, we focus on the cellular origin of the conduction system components, the molecular-genetic mechanisms that control their differentiation, and their impact on arrhythmias.

**Origin and Composition of the Developing Conduction System**

The distinct components of the cardiac conduction system of the heart are essentially myocardial (Figure 1).1-4 The hypothesis that the conduction system may have a neural crest origin has not been supported by evidence. Retroviral labeled single cardiac cells of the early embryonic chicken heart (HH15-17, ±30 somites) were found to give rise to multicellular clones containing both working myocardium and myocardial cells of the conduction system.5,6 Moreover, in both chicken and mouse, labeled neural crest or proepicardial cells were never traced in the conduction system components, even though their descendants were found in close association with these components.5

The conduction system components are innervated by cardiac ganglia largely derived from the neural crest.7,8 A large number of fibroblasts are found within the mature conduction system, and the sheaths that, in the human heart, insulate the ventricular pathways.3,4,9 These fibroblasts are derived from the epicardium, endocardium, and neural crest. Although the nervous and fibrous tissues are important for formation and function of the conduction system,10 the cardiomyocytes are the essential cells for the generation and propagation of the impulse. In the embryo, the conduction system is not innervated and interstitial fibroblasts are sparse or absent within the myocardial components of the system.4,11,12 It is the cardiomyocyte, therefore, which is the source and principal cell type of the conduction system.

**The Conduction System Is Evolutionary Conserved as an Integral Component of the Building Plan of the Heart**

The conduction system is not a single system of more or less identical cells, but each component is distinctive in terms of molecular phenotype, morphology, and function. All myocytes within the conduction system, nonetheless, share some properties that distinguish them from working myocardium, such as poorly developed sarcomeres and sarcoplasmic reticulum, sparse mitochondria, and other features.1,7,13 Recent advances in molecular genetics have revealed that distinctive regulatory pathways are responsible for their specification and formation. Below, we will discuss the formation of each component separately.

To understand the development of the conduction system, we must first consider the anatomic arrangement of its components in the formed heart. The SAN, located at the junction of the superior caval vein and right atrium, is the primary pacemaking component that generates the electric impulse.4-14 From there, the impulse propagates rapidly through the atrial muscle and is slowed in the AVN. The node is located in the floor of the right atrium, occupying the apex of the triangle of Koch.9 Via the penetrating atrioventricular bundle, this slowly conducting node forms the only myocardial connection between the atria and ventricles. Elsewhere, these muscle masses are electrically insulated one from the other by the fibroadipose tissues of the atrioventricular junctions (Figure 1). The delay in propagation of the impulse allows the ventricles to be diastolic during atrial contraction and provides protection from ventricular arrhythmias which may be triggered by the atria.15,16 The electric impulse then enters the fast-conducting AVB and BBs to reach the PVCS, which rapidly transmits the impulse to the working myocardium so that the ventricular mass is activated from apex to base. It is this anatomic
configuration that underscores generation of the ECG, in which the atrial activation shows as the P-wave, the AV delay as the P-Q interval, and the ventricular activation as the QRS complex (Figure 2).

The components of the adult mammalian conduction system are morphologically well defined, although species differences exist. Hoofed animals have well-developed structures, rodents have poorly-developed ones, and human is somewhere in between. Also, in birds the system is well-developed. In lower vertebrates, or mammalian embryos, this is not the case. The activation and contraction patterns, and the ability to generate an ECG as described above, nonetheless, are evolutionary conserved, as has been realized already in the fish heart. In chicken, the “mature” ECG can be derived as soon as ventricular and atrial chambers develop (Figure 2; HH stage 13, 20 somites). When the heart tube forms in the early embryo, pacemaker activity can be recorded at the venous pole. The electric impulse migrates very slowly through the myocardium, resulting in a sinusoidal ECG and a peristaltic contraction pattern. Atria and ventricles will subsequently form at discrete sites of the heart tube. They express Cx40 and Cx43, which form high-conductance gap-junction channels, and the \( \alpha \)-subunit of the cardiac sodium channel Nav1.5 (encoded by \( Scn5a \)), providing these compartments with high conduction velocity.

By contrast, a slow-conducting AVC positioned between the atrial and ventricular chambers, negative for Cx40 and Cx43, but positive for Cx30.2 and Cx45, which form low-conductance gap-junction channels, slows still further the impulse, and is “left behind” to function so as to delay the depolarizing impulse. The configuration of the distinct components of the embryonic heart, or the “cardiac building plan,” is sufficient to maintain an activation cycle, with the derived ECG resembling the adult pattern. These observations imply that the function provided by a conduction system is present already in the early embryonic heart, and that, like in lower vertebrates, this function does not require the formation of morphologically defined components.

**Mechanism and Timing of the Specification of the Conduction System Components**

The different components of the definitive system are formed “on the spot” from nonmigrating myocardial precursor cells present at a particular location within the developing heart. Thus, the developmental path taken by a given myocyte depends on its position in the developing heart. The development of a distinct conduction system component from its precursor can be modeled as a simple decision process, in which a precursor cell will turn into either a “conducting” or a working cardiomyocyte. Molecular genetic studies have revealed the functional requirement of a network of transcription factors for the formation of the sinus and atrioventricular nodes and the rapidly conducting ventricular pathways. These include broadly expressed factors of the core cardiac transcriptional network, such as Tbx5 and Nkx2-5, and the transcriptional repressors Tbx2, Tbx3, and Id2, which are expressed specifically in the precursors of these conduction

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### Figure 1

**Figure 1.** Schematic overview of heart development in higher vertebrates. The early heart tube has a primitive phenotype (light purple). Chamber myocardium (gray) expands from the outer curvatures of the primary heart tube, whereas nonchamber myocardium (purple) 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), atrioventricular canal myocardium to the atrioventricular node (avn) and atrioventricular junction (avj). The ventricular septum crest part of the primary ring (pr) will form the atrioventricular bundle (avb). First 3 panels show left-lateral views. ev indicates embryonic ventricle; a, atrium; r/l a, right/left atrium; r/l v, right/left ventricle; scv, superior caval vein; r/lbb, right/left bundle branch; pvcvs, peripheral ventricular conduction system.

### Figure 2

**Figure 2.** Development of chambers and ECG. A, Left-sided view of embryonic hearts of embryonic day (E) 8 to 8.5 and E9.5 to 10.5, respectively. Formation of the chambers is in accordance with the development of an adult-like ECG. Red arrows demarcate the ventricular and atrial inner curvatures. B, Schematic overview showing the different ECG segments correlating with the different components of the embryonic heart. The white arrow depicts the flow through and the sequential mode of activation. Note the PQ interval that is caused by the slow conducting atrioventricular canal (avc). C, Serial sections of an E9.5 mouse heart. Cx40 is expressed in the developing chambers, whereas Tbx3 demarcates the AVC (black arrow). Expression of Cx40 is conserved in the developing (E3) chicken heart.
system components.24–26 These repressors maintain the phenotype typical for the conduction system, and in their absence, the precursors differentiate into working myocardium, which seems to be the default developmental path.27

Particularly important questions are, when do cells decide to become working cardiomyocytes, or become committed to the conduction system, and what is the mechanism underlying this decision? Three models have been proposed for formation of the conduction system. These are the “multiple ring theory,” the “recruitment model,” and the “early specification model” (Figure 3). The multiple ring model hypothesizes rings of “conduction system tissue” in the tubular heart (reviewed in10). When the chambers develop and expand, the flanking tissue of the sinus venosus, AVC, primary (interventricular) ring, and outflow tract proliferate much less compared to the expanding atrial and ventricular chambers,27–29 thus forming relative constrictions. These regions express markers such as HNK1 and engrailed-2-/lacZ (also known as CCS-lacZ).10,30 The “rings” are supposed to be present already in the initial tubular heart, but no evidence in terms of expression patterns or structures has yet been produced to validate this notion. Indeed, recent work has shown that the tubular heart itself contains little more than the precursors for the left ventricle.31 It is impossible, therefore, that the rings unequivocally present in the heart subsequent to looping and ballooning of the atrial and ventricular chambers correspond to the postulated rings in the tubular heart.

Pioneering lineage analyses in chicken by the Gourdie and Mikawa labs demonstrated that, in the chicken, single myocardial cells infected at embryonic day (E)3 (HH15-17) had formed, by E14-20, cell clusters in the central conduction system (right AV ring bundle, AVB). These clusters were marked by EAP-300 expression, and extended into adjacent EAP-300-negative working myocardium, demonstrating that working myocardium and conduction system cells are derived from a common progenitor present at the stage of infection.5,6 Their interpretation that the components of the conduction system form by inductive recruitment of myocytes to an initial conduction system framework, which can be summarized as the recruitment model,23,32 however, deserves additional comment. First, the initial framework for the AV conduction system as seen in the early embryo was postulated, but not defined. Indeed, inductive signals from this framework have yet to be demonstrated or identified. Second, the myocytes making up the conduction system were postulated to be quiescent, necessitating growth by recruitment of adjacent myocytes not from the conduction system. Recent studies, however, have demonstrated that myocytes with the phenotype of the conduction system proliferate at a slow rate.28,29 Third, the expression of EAP-300 was used as criterion for a conduction system cell in these retrospective labeling experiments. If there was truly recruitment to the conduction system, the labeled precursor cells should be negative for this marker. All embryonic cardiomyocytes, nonetheless, express EAP-300 until this marker is downregulated in the working myocardium late in development to become restricted to the conduction system.33 It is more likely, therefore, that in the chick embryo, EAP-300-positive cells were labeled, the progeny of which either maintained EAP-300 expression to form the AV conduction system, or lost EAP-300 expression to become recruited to the working myocardium. In other words, the data can be interpreted equally well to support the notion of early specification.
This view, originating from our laboratory,\textsuperscript{13} states that the precursors of the conduction system are specified early in heart development, proliferate slowly, and develop further into the conduction system components.\textsuperscript{13} In birds, and various mammalian species, the putative precursors specifically express marker genes from early stages onwards\textsuperscript{24,30,34–39} (Figure 4A and 4B; Table). The key issue is again whether or not marker-negative myocytes will initiate

| Table. Markers of the Distinct Components of the Mouse Conduction System |
|--------------------------|----------------|----------------|--------|--------|--------|----------------|--------|--------|--------|
| Gene | SAN | AVC | AVN | Early | Late | BB | Trab myo | PVCS | Early | Late | PV | A | V | OFT |
| Cx40 | – | – | – | – | + | + | + | – | + | + | + | – | – | – |
| Cx43 | – | – | – | – | – | + | + | – | + | + | + | + | – | – |
| Nppa | – | – | – | – | – | + | + | – | – | + | + | – | – | – |
| Hcn4 | – | – | – | – | – | – | + | – | + | – | – | – | – | – |
| Tbx3 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Tbx2 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Mx2 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Tbx18 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Shox2 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Tbx5 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| Nkx2–5 | – | – | – | – | – | + | + | – | + | + | + | + | – | – |
| Id2 | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| CCS–lacZ | – | – | – | – | – | + | + | – | – | – | + | + | + | + |
| minK–lacZ | – | – | – | – | – | + | + | – | – | – | – | – | – | – |
| cGata6–lacZ/Cre | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

The table shows the currently most useful markers to distinguish the components of the developing conduction system from other myocardium. Markers not sufficiently specific or initially broadly expressed, not clearly negative in unmarked regions, or not sufficiently characterized during development, have been omitted. Trab myo indicates trabecular myocardium and includes the early ventricular wall before a clear compact wall has been formed; SH, sinus horns; PV, pulmonary vein myocardium; A, atrium; V, ventricle; OFT, outflow tract.

1. Expression at low levels in all cardiomyocytes, upregulation in the AVCS and PVCS late in development.
2. Downregulated during late fetal stages.
3. Expression in early right ventricle has been observed.
4. Expression limited to SAN head.
5. Initially expressed in the LV, low in RV, thereafter restriction to AVB, BB, Trab.
7. Expressed in the right and left atrial working myocytes.
8. Expressed in right atrium and left ventricle, not entire AV positive.
marker expression, thus being recruited to the conduction system lineage, or vice versa. In mouse, the region in which the conduction system cells develop (the initial framework) expresses Tbx2, Msx2, or Tbx1. From approximately the same stage onward (E8.5 to 9.5) the adjacent Tbx2/Msx2/Tbx3-negative myocytes initiate expression of chamber differentiation markers Nppa, Cx40, Cx43, or Smx. Myocytes not expressing a marker from these groups, or coexpressing a marker from both groups, are very rare (Figure 4B; Table), indicative of a binary decision of myocytes to follow the path of development into either the myocytes making up the conduction system, or working myocytes. Using the Cre-loxP system in mouse, the distinctive cell types can be labeled based on their expression of either conduction system markers or chamber (working)-myocardium-specific genes. Such an analysis revealed that, in mouse embryos, Nppa-positive atrial cells were not recruited to the Tbx3-positive SAN, and that the nodal cells, after being specified by their expression of Tbx3, grow on their own. Analysis of a recently established line, in which Cre was inserted into the Tbx2 gene, thus allowing to label and follow Tbx2-positive primary myocardium, revealed that the AV conduction system was composed of progeny of initially Tbx2-positive cells within the myocardium of the atrioventricular canal (our unpublished observations). Also, a large part of the Cx40/Cx43-positive left ventricle was also found to be derived from the Tbx2-positive cells initially making up the atrioventricular canal. These data support a model in which cells expressing at an early stage markers of the conduction system either defy differentiation to the working phenotype, allowing them to form the components of the conduction system, or loose their markers as myocytes within the conduction system and differentiate into working myocardium (Figure 3C).

Pacemaker Activity, Polarity, and the Formation of the SAN

Normally, the cardiac cells within the SAN, positioned at the inflow of the right atrium, have the highest pacemaker activity. This structure, therefore, dictates the rhythm of contraction. In the embryo, the heart is a simple tubular structure consisting of embryonic, or primitive, myocardial cells with an endocardial lining. All myocytes have pacemaker activity, but the cells at the most proximal part have the highest intrinsic rate, and, therefore, function as the dominant pacemaker that dictates the heart rate (reviewed in 13,27). This localized electric activity emerges even before the embryonic myocytes are able to contract20 and is therefore the first function of the heart to arise.

Recent genetic lineage and molecular analyses have greatly advanced our insight into the formation of the SAN (Figure 5A). The causal-most portion of the primitive heart tube expresses the pacemaker channel Hcn4, which is required for pacemaker activity in the mouse embryo and is likely responsible for the caudal localization of dominant pacemaker activity in the early heart tube. Subsequent to the formation of this heart tube (mouse E9.5), its inflow tract forms the atria, while Tbx18-expressing mesenchymal cardiac progenitor cells are added to the tube to form the sinus horns and eventually the SAN. Dominant pacemaker activity is always found at the venous pole, indicating that it shifts to these newly added cardiac cells at the venous side. During its differentiation, the sinus myocardium initiates the expression of Tbx3 and Hcn4, but not the cardiac homeobox transcription factor Nkx2-5. Nkx2-5 remains expressed in the “older” myocardium of the primary heart tube, now turned into the developing atrium, where it activates Cx40 and suppresses Hcn4 and Tbx3. Thus, the repressive activity and restricted expression of Nkx2-5 provides a mechanism for how pacemaker activity becomes progressively relegated to the most recently added components of the venous pole of the heart.

Early in development, the entire sinus venosus, including the primordium of the sinus node, acts as pacemaker. Subsequently, pacemaker activity will be confined to the node itself. The precursors of the definitive node, recognized as a Tbx3-expressing Nkx2-5–negative subdomain within the Hcn4-positive sinus venous, forms from after E9.5 in mouse, shortly after the establishment of the Nkx2-5–positive atria (Figure 5A). During the fetal period, the sinus horns obtain an atrial myocardial phenotype, with upregulation of Cx40 and Cx43 and downregulation of Hcn4. They will form the systemic venous sinus of the right atrium and the myocardial sleeves of the superior and inferior caval veins, along with the remnant of the left caval vein, which becomes the coronary sinus in the human. The original pacemaker gene program (Hcn4-positive, Cx40/Cx43-negative) and thereby probably the dominant pacemaker activity, becomes confined to the Tbx3-expressing SAN subdomain within the systemic venous sinus. Tbx18, Tbx5, Nkx2-5, Tbx3, Shox2, and Pitx2c are involved in the formation of the sinus venosus and SAN, and in confinement of the pacemaker gene program to the SAN at the junction of the right atrium and superior caval vein (Figure 5A). The extent to which the sinus venosus matures to atrial working myocardium, and the underlying mechanisms, are not known. How these regulatory pathways link to progressive sinus nodal dysfunction, which involves changes in expression of ion channel genes, changes in tissue composition such as increased deposition of connective tissue, and possible loss of nodal cells, remains to be defined.

Development of the Atrioventricular Conduction System and Atrioventricular Conduction Defects

The atrial component of the AV conduction system comprises the AVN in the adult, along with the right AV ring bundle around the tricuspid valve and the retroaortic root branch in birds and mammalian embryos. The atrioventricular bundle, also an integral part of the AV conduction system, will be discussed separately. The majority of studies support the view that it is the embryonic AVC that contains the precursors of the AVN and the AV ring bundle(s) (Figure 1).

The myocardium of the embryonic tube has a primitive phenotype that displays slow transmission of the electric impulse (Figure 2A). While the heart tube further elongates by adding cardiac progenitors to its poles and dorsal wall, the primitive heart tube regionally differentiates into fast conducting and contracting ventricular and atrial cham-
ber myocardium, while the myocardium of the AVC and outflow tract retains its original embryonic phenotype (Figures 1 and 6). Owing to the pacemaker activity at the venous pole, and the delay of the impulse in the AVC, the sluggish contraction of the embryonic tube is converted into a pattern of rapid serial contraction of the atrial and ventricular chambers (Figure 2A). Chamber differentiation, and local prevention of differentiation, therefore, are key events in the local formation and function of the AVN, as is the contribution of inputs from the developing primary atrial septum.

There is considerable insight into the molecular mechanisms underlying AVC differentiation (Figure 6). One of the earliest events is the expression of the extracellular signal molecule Bmp2 in the embryonic precursors of the AVC. Bmp2, acting through type-I BMP receptors (Alk3), activates Tbx2. Tbx2, together with Tbx3 and Msx2, prevents expression of chamber-specific genes including the T-box factor target genes Nppa, Cx40, Cx43, and Scn5a in the AVC/AVN. Tbx20 and a Bmp2-Tbx2-Notch-Hey1/2 signaling cascade55,56 regulate Tbx2 and delimit the AVC domain. This pathway is also found in zebrafish, indicating evolutionary conservation of AVC formation. Tbx5 and Nkx2-5 are expressed broadly in the heart and are involved in the formation and maintenance of the conduction system. Dominant mutations in these genes cause congenital heart defects and conduction system abnormalities, including atrioventricular block, in adult humans and mice.58–64 The electrophysiological defects can occur in the absence of cardiac structural malformations, suggesting that Tbx5 and Nkx2-5 have roles in conduction system development independent of their roles in cardiac morphogenesis. A gain of function mutation in TBX5 leads to Holt-Oram phenotype and paroxysmal atrial fibrillation.65 Mice with only one functional copy of Tbx5 display postnatal failure in maturation of the AVC component, as visualized by persistent minK-lacZ expression, a marker for this component of the conduction system.63 The mechanism that leads from haploinsufficiency of these factors to the localized AV conduction defects is unclear. Tbx5

Figure 5. Development of the SAN and AVB. A, Model for SAN and sinus horn formation in the right wall of the venous pole. Yellow shows mesenchyme, purple nodal myocardium, gray “chamber-type” myocardium. Depicted transcription factors regulate formation of boundaries and zones of expression and differentiation of atrium, SAN, and cardinal vein. raw indicates right atrial wall; rvv, right venous valve; scv, superior caval vein; WM, working myocardium. B, Model for AVB and BB formation. Serial section of a wild-type embryo, stained for Tbx3 and Cx43, in complementary patterns in the ventricular septum (versus). In Tbx3 knock-outs (KO), the atrioventricular bundle (avb, arrow head) and bundle branches (bb) ectopically express Cx43. Depicted factors control formation of the avb and ventricular working myocardium, respectively.
and Nkx2-5 are essential activators of the chamber genes important for conduction, and interact on the genetic and physical level with several classes of transcription factors, including T-box factors Tbx2 and Tbx3.64,66,67 Moreover, Tbx5 is a crucial activator of Tbx3.66 These observations suggest that imbalance between these transcription factors may affect their interactions and function, specifically in the AVC and AVN.

Several arrhythmias originate from the AV junction. Late in development, expression of Tbx3, minK-lacZ, and other markers disappears from most of the AVC, becoming confined to the AVN,24,38,63 and indicating disappearance of AVC myocardium, either by apoptosis or differentiation into working myocardium. Remnants of embryonic nodal AVC myocardium, nonetheless, surround the AV valves in the working myocardium. Remnants of embryonic nodal AVC myocardium, either by apoptosis or differentiation into working myocardium, allow the electric signal to bypass the normal connection through the atrioventricular conduction axis.64 Furthermore, inhibition of epicardial formation results in disturbed formation of the fibrous insulation and prenatatal ventricular preexcitation.73 These data provide evidence for the importance of the fibrous insulation to prevent peri- and postnatal ventricular preexcitation arrhythmias.

**Formation of the Atrioventricular Bundle and Proximal Bundle Branches**

The AVB is generally regarded as a component of the AV conduction system. Indeed, the myocytes of the AVB and BBs are more primitive than working myocytes, much resembling the phenotype of the AV nodal myocytes.7,11 Consistently, the AVB and BBs can serve as pacemaker for the ventricles in case of AV block. In contrast to the AVN, however, they conduct the impulse very rapidly, and their myocytes are well coupled by gap-junctions. Cx40 is essential for the fast rate of impulse propagation through the AVB and BBs.16 In mammals and chicken, Cx40 serves as a very useful and specific marker that distinguishes the fast conducting continuity between the chambers, with the ventral and dorsal aspects of the AVC being preferential sites of conduction.22,69 After septation, fibrous tissue will form from the AV cushion and epicardial mesenchyme and physically separate the atria and ventricles. The only connection that remains is the AVB, which connects the AVN at the atrial side of the fibrous body with the ventricles. The mechanisms that underlie the formation of this fibrous separation, and the confinement of the AVC tissue to the AVN, have not been established in detail.

AV reentrant tachycardias are caused by the presence of abnormal accessory myocardial bundles connecting the atrial and ventricular myocardium.70 Usually, these bundles conduct rapidly, as in the Wolff-Parkinson-White (WPW) syndrome, suggesting their myocytes retain a working myocardial phenotype. Accessory bundles with AV-nodal properties, as found in Mahaim tachycardia, are rare, and usually occur at the tricuspid side, where normally a more pronounced AV nodal phenotype is present.71 The mechanism underlying the formation of accessory bundles probably involves abnormalities in both the formation of the fibrous insulation and the differentiation to fast-conducting working myocardium of the original slow-conducting nodal AVC myocardium. When BMP signaling was disrupted in the AVC through cGata6-Cre–mediated inactivation of Bmpr1a/Alk3, the type IA receptor for BMP, the insulation was disrupted, a myocardial AV connection expressing Cx43 was present that was sufficient to cause preexcitation, and AVN morphology was changed.51,72 BMP signaling in the AVC is essential for the expression of Tbx2, which together with Tbx3 suppresses working differentiation and Cx40/43 expression (Figure 6). It is also essential for the formation of the AV cushions,72 an integral component of the fibrous insulation. Hence, the BMP–Tbx pathway in the AVC itself may play an important role in coordinating the processes involved in AV insulation.

Mutations in PRKAG2 have also been associated with ventricular preexcitation, specifically the WPW syndrome. Expression of a mutant isoform leads to disruption of the fibrous insulation and direct contact between the atrial and ventricular myocardium, allowing the electric signal to bypass the normal connection through the atrioventricular conduction axis.69 Furthermore, inhibition of epicardial formation results in disturbed formation of the fibrous insulation and prenatal ventricular preexcitation.73 These data provide evidence for the importance of the fibrous insulation to prevent peri- and postnatal ventricular preexcitation arrhythmias.
components (AVB, BBs, PVCS) from the Cx40-negative slow-conducting AV conduction system components (AVN) and working myocytes of the ventricle. Some details regarding the molecular mechanism of AVB and BB formation are shown in Figure 5B. Several of these factors have been implicated in AVB and BB dysfunction in human and mouse.

A variety of origins of the AVB have been proposed (reviewed in ), but general consensus is that the AVB forms from the crest of the developing ventricular septum. From the outset, it is in direct contact with the developing AVC/AVN. With further development of the ventricular septum, left and right BBs develop directly from the subendocardial myocardies and bifurcate from the AVB. The AV conduction system and AVB are formed from a network of precursors recognizable early in development (4 to 5 weeks human, rat E11, mouse E9.5) by a set of marker genes (Table). These cells represent the interventricular ring and subendocardial myocardial cells draping over the developing ventricular septum, which includes the putative AVN primordium, right AV ring bundle, AVB primordium, and BBs (Figures 1 and 4). To date, the mechanism controlling the early highly localized expression of these markers has not been defined.

The myocytes of the AVB and BBs are distinctive from the myocytes of the AVC/AVN. Compared to the latter, AVB development is much more sensitive to haploinsufficiency of combinations of Tbx5, Nkx2-5, and Id2 to or loss of Tbx5. Moreover, recent lineage analyses from our laboratory, as yet unpublished, indicated that the AVC is derived from daughter cells of Tbx2-expressing progenitors, whereas the AVB and branches are derived from cells that never expressed Tbx2, suggesting segregation of the lineages of these components as early as E8 in mouse.

Based on functional studies, the AVN and ventricular conduction system components have been suggested to form as independent modules that connect subsequent to their formation. Therefore, although specified AVB and BBs are present from the outset (see previous section), they may initially not be functionally equipped to rapidly propagate the electric impulse. This was confirmed in Tbx3-deficient fetuses in which the AVB and BB adopt the working myocardial phenotype but do not show any changes in AV conduction times or ventricular activation pattern. Consistently, the AVB does not express Cx43 (Figures 4B and 5B) and in both chicken and mouse activates Cx40 expression only at midfetal stages (Figure 5B). This suggests that, in the early embryonic heart, the preferential pathway for the impulse is from the AVC directly through the trabecules that do express Cx40 and Cx43 from the onset of their formation (mouse E9.5) and that are directly connected with the AVC myocardium (see Figure 2C). Indeed, in mouse fast ventricular activation was observed at E9.5, starting from the slow conducting dorsal AVC wall (AVN anlage). By the time the entire AVB expresses Cx40, the atria and ventricles have become electrically insulated by formation of a connective tissue layer, leaving the now Cx40-positive AVB as the only, rapidly conducting, myocardial connection between the AVN and the ventricles.

Distal Bundle Branches and Peripheral Venticular Conduction System

In the mature heart, the distal BBs and PVCS are only one to a few cells thick, and are located directly below the endocardium. In chicken, an additional periarterial network of Purkinje-like cells is present. Together, these components form the PVCS, characterized by Cx40 expression but otherwise an embryonic primitive phenotype.

How does the PVCS develop? Mammalian and chicken embryonic hearts display fast ventricular conduction at stages when trabeculations have just been formed. Also in hearts of lower vertebrates, which do not possess a distinct PVCS network, the ventricle is activated from apex to base, indicating that the substrate for preferential conduction toward the apex is already present. The spongy trabecular myocardium of the fish heart is remarkably similar to that of mammalian and chicken embryos. These observations indicate that the trabeculations are the functional and structural precursors of the PVCS. In chicken and mouse, Cx40, TASK-1 (Kcnk-3), Nppa/b (ANF/BNF), and EAP-300 (chicken) are expressed transmurally in the embryonic ventricles. The expression of these markers becomes gradually restricted to the trabeculations and, subsequently, the PVCS, concomitant with the formation of the nonexpressing compact layer at the epicardial side (Figure 7). The compact zone rapidly enlarges while the trabecular Cx40-positive zone remains more or less constant and thus decreases in relative terms. After birth, a further Nkx2-5-dependent maturation step takes place, in which the trabecular zone is remodelled into the definitive subendocardial PVCS of only 1 to a few cells thick. Together, these observations suggest that the process of PVCS formation involves differentiation at the epicardial side of immature embryonic ventricular cells into Cx40/Nppa/TASK-1-negative compact working myocardium, whereas at the endocardial side, the immature (PVCS-like) trabecular Cx40/Nppa/TASK-1-positive phenotype is maintained in myocytes that form the PVCS network of the formed heart (Figure 7).

Neuregulin signaling is necessary and sufficient for the formation of trabeculations. The results indicate that neuregulin signaling regulates the relative proportion of the embryonic ventricular wall that forms trabecular as opposed to compact myocardium, implicating a role in PVCS formation. During the formation of the trabeculations, Notch signaling was found independently to regulate ventricular cardiomyocyte proliferation and differentiation, acting through Bmp10 and independently through ephrinB2 and neuregulin signaling.

Endothelin signaling plays a role in the formation of chicken periarterial and subendocardial Purkinje cells. Both endothelin-1 (ET-1) and endothelin converting enzyme (ECE-1) were shown to be required for the induction of the PVCS phenotype in ventricular cells in vitro and in vivo. ECE-1 is preferentially expressed in endocardial cells and endothelial cells of the coronary arteries, indicating a mechanism for the timing and location of PVCS formation. The sequence of activation of the ventricles shifts from a unidirectional pattern to a mature apex-to-base pattern around E7–9 (HH29–35). This change has been attributed to the
maturation of the AVB-PVCS system, although, as discussed in the previous section, it may involve the late functional maturation of only the AVB. Changes in hemodynamic load were found to alter the timing of this shift, indicating that biomechanical forces affect the development of the ventricular conduction system. ECE-1 expression and Cx40 were also found to be regulated by hemodynamic load, correlating with the changes in timing of the shift to the mature apex-to-base pattern. These data suggested that biomechanical forces, such as shear stress and pressure, acting through stretch/pressure-induced ET and ECE-1, play a role in the development of the ventricular conduction system in chicken. Together, these data indicate a mechanism of local induction of PVCS phenotype in ventricular myocardium, but how this fits with the model of maintenance of the PVCS phenotype described above is not yet clear. Evidence for the role of ET-1 signaling in mammalian PVCS formation is largely lacking, and a functional PVCS-like trabecular system is present before the onset of high pressure circulation, suggesting hemodynamic factors are not essential for PVCS formation. A model for PVCS formation incorporating both views involves neuregulin signaling-mediated induction, formation and maintenance of the early trabecular ventricular phenotype, and endothelin signaling-mediated reinforcement and maintenance of the PVCS phenotype in the subendocardial myocytes later in development (Figure 7).

Why Are Some Regions of the Heart More Arrhythmogenic Than Others?

Nodal Remnants

A scheme of the cardiac building plan is shown in Figure 1. Initially, the primary heart tube consists of poorly coupled muscle cells, with highest pacemaker activity at the venous pole, displaying sinusoidal depolarization waves and matching peristaltic contractions. Such a heart does not need valves, nor a distinct conduction system. When the tube lengthens by recruiting progenitors to its poles, chambers develop regionally that consist of fast-conducting muscle cells, allowing for efficacious contraction. Owing to the regional action of a transcription factor network of inductors, activators, and repressors, the forming chambers remain flanked by primary myocardium, the sphincteric action of which substitutes valve function. These flanking regions can be considered as the “embryonic conduction system,” although not all their cells will remain as the definitive conduction system. The forming ventricular trabecular myocardium consists of well-coupled cells that will form the PVCS. Taken together, such a building plan of the embryonic heart allows for the derivation of an ECG in absence of a morphological conduction system. The pattern of expression of Tbx3 in the embryonic heart, as presented in Figure 4, may betrays a developmental base for the regions in the formed heart that are more prone to arrhythmia than other regions which form the chambers. The orifice of the coronary sinus originates from the orifice of the embryonic left caval vein, the terminal crest or internodal myocardium originates from the floor of the embryonic atrium, and the lower rim of the atrium, in other words the myocardium surrounding the tricuspid and mitral valves, is derived from AVC myocardium. These regions display significantly more tachycardia and rhythmical anomalies than other parts of the atrium (eg,92). It is tempting to speculate that, under pathological conditions such as gene defects or anatomic substrates, the embryonic origin of these tissues
becomes apparent, because this myocardium has insufficiently lost its properties to act as pacemaker.

Concepts for the existence of so-called internodal tracts have always been based on the patterns of expression of markers such as HNK-1, CCS-lacZ, or Tbx3 (Figure 4D).24,36,77 The phenotype and function of the myocytes within such postulated tracts, their significance for conduction system function, or even their relation to the conduction system, has not been revealed. If such myocytes have any function apart from that of a working cell, they will probably resemble the slowly-conducting remnants of the sinus venosus that have been incorporated into the right atrium and that normally are functionally insignificant.

Another region that is prone to life-threatening arrhythmias is the right ventricular outflow tract.93 The embryonic outflow tract has been proposed as an extension of the developing conduction system because it initially retains the embryonic phenotype and expresses markers such as mink-lacZ and Cx45 that have been associated with the conduction system.38,94 With normal development, the proximal embryonic outflow tract will largely differentiate into right ventricular working myocardium.95 Persistence of the embryonic phenotype may be at the base of triggered automatic arrhythmias, as recently reviewed.96 The most distal part of the embryonic outflow tract will disappear by apoptosis.95,97 If this process fails, it can easily be imagined that persisting myocytes above the arterial valves can cause ventricular tachycardias.93

**Pulmonary Myocardium**

The pulmonary venous sleeves have become well-established, and intensely studied, sources of arrhythmias. The mechanisms underlying the initiation and maintenance of arrhythmias vary from reentry, enhanced triggered afterdepolarizations, to ectopic pacemaker activity.98 If the myocytes within these sleeves would be a component of the developing conduction system, insights into node and conduction system development would contribute to our understanding of the underlying arrhythmogenic mechanisms. The expression in the left atrial myocardium of markers associated with the components of the conduction, such as CCS-lacZ, has been taken by some authors to suggest that the pulmonary venous sleeves are a component of the developing conduction system (reviewed in98). But, although CCS-lacZ marks the AVB, BBs, and PVCS,30 it does not precisely mark the components of the atrial conduction system. It is also active in atrial working myocardium, and absent from the SAN.30,81,99,100 Hence, in our opinion, pulmonary expression of these markers, which do not have a known functional significance for myocytes having a conduction system phenotype, does not provide sufficient evidence to support the notion that this myocardium is a component of the conduction system.

The pulmonary venous myocardial sleeves have electrophysiological properties distinctive from the atrial myocardium, which possibly contributes to their arrhythmogenic activity.98 Moreover, ectopic pacemaker activity has been observed within the sleeves, and some have suggested that they contain “nodal”-like cells (reviewed in102), albeit that much evidence exists to counter this suggestion. Developmental genetic and lineage studies, moreover, have indicated that the myocardium of the pulmonary venous sleeves, and the sinus venosus myocardium, have different origins, and are regulated by different regulatory pathways.49,101 On its differentiation from mesenchymal progenitor cells, the pulmonary venous myocardium immediately activates genes associated with atrial working myocardium that are responsible for fast conduction, including Cx40, Cx43, and Scn5A, whereas pacemaker channel gene Hcn4 is hardly expressed. This contrasts to the gene program of the sinus venosus, including the SAN. Thus, whereas all conduction system components, and their associated regions, are characterized by a certain degree of suppression of working myocardial properties and retention of some properties suggestive of a conductive phenotype, the myocardium making up the pulmonary venous sleeves readily differentiates to atrial working myocardium immediately on its formation. The strong reduction of Nkx2-5 levels results in upregulation of Hcn4 and downregulation of Cx40 in the pulmonary venous myocardium, suggesting that Nkx2-5 may help to establish the working phenotype and suppress the conduction system phenotype.101 Furthermore, Pitx2c is required for the differentiation of the pulmonary venous myocardium from its progenitors.101 Pitx2 is located in proximity to 2 sequence variants on chromosome 4q25 strongly associated with atrial fibrillation,102 suggesting the possibility that variations in Pitx2 expression may influence the pulmonary venous myocardial phenotype.

**Conclusions**

We have discussed the development of the conduction system, emphasizing current insights into the molecular mechanisms and lineage relationships, and tried to present a logical framework and open questions. This framework may provide a basis for the development of rational strategies to repair or regenerate the conduction system, for example by (re)programming cells into the desired cardiac subtype in situ or ex vivo.

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**Disclosures**

None.

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