Development and Structure of the Cardiac Conduction System

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A major function of the heart is to propel blood, by mechanical contraction of the cardiac muscle, through the vascular (arterial and venous) system. To achieve this task, the underlying electrical conduction system generates an electrical impulse that sequentially propagates from the sinus node through the atria, the atrioventricular node, and His-Purkinje system to the ventricles. This process initiates electromechanical engagement or coupling, producing myocardial contraction.

This chapter will review the embryology and development of the conduction system, outlining known transcriptional regulators and signaling pathways that support formation and differentiation of the specialized cells the conduction system and will discuss how disorders of development lead to abnormalities of conduction and to clinical arrhythmias. In addition, the chapter will review the anatomy of the heart with focus on the structures that support the electrophysiologic properties of the conduction system as well as describe the conduction system abnormalities associated with specific congenital cardiac defects.

Part I—Development of the Conduction System

It is not a surprise that the cardiac conduction system develops in concert with the structural maturation of the heart itself (Fig. 1.1). The heart forms early in embryogenesis as cardiac cells originate in the epiblast, which is lateral to the primitive streak. From here, cardiac cells migrate in a rostrolateral direction to bilateral areas of a lateral plate mesoderm. The lateral mesoderm separates into somatic and splanchnic epithelial layers. The bilateral splanchnic mesoderm will generate cardiac precursor cells and is referred to as the primary heart field. It is a subset of these cells that migrate towards the midline and fuse to form the primary myocardial heart tube. The heart tube is lined by an outer layer of cardiac jelly which is in turn surrounded by differentiating mesoderm from the primary heart tube which will form the myocardium. During further development of the heart tube, additional cells from the splanchnic mesoderm, from the caudal portion of the secondary heart field, will continue to contribute to the dorsal (venous) pole of the heart. Progenitor cells in the pharyngeal mesoderm and the remainder of the secondary heart

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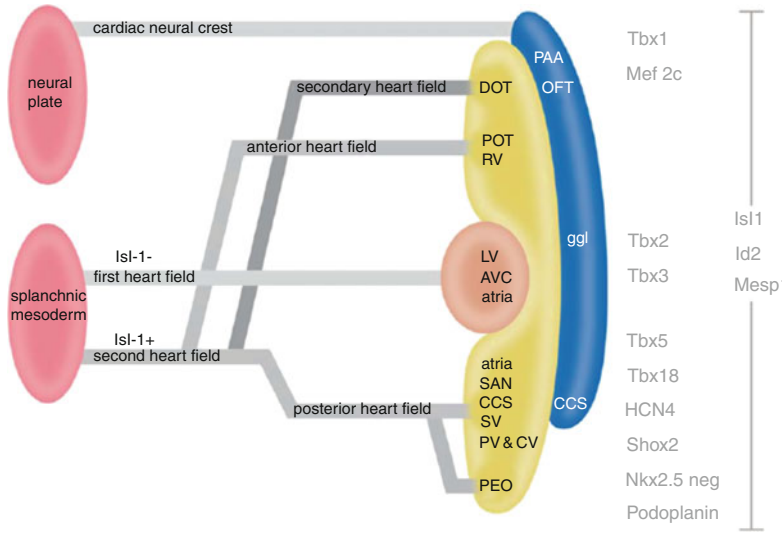

Fig. 1.1 Schematic representation of the primary heart tube (*brown*) and the secondary added myocardium derived from the second heart field (*yellow*), including differential genes and proteins expressed in the second heart field. The second heart field can be divided into an anterior heart field and a secondary heart field at the anterior pole of the heart, and a posterior heart field at the venous pole of the heart. At the venous pole of the heart, the proepicardial organ (PEO) is also derived from the posterior heart field, and is the source of the epicardium and epicardium-derived cells. Neural crest cells (depicted in *dark blue*) migrate to the heart and enter the heart at both the arterial and venous pole. *AVC* atrioventricular canal, *CV* cardinal veins, *CCS* central conduction system, *DOT* distal outflow tract, *LV* left ventricle, *OFT* outflow tract, *PAA* pharyngeal arch arteries, *POT* proximal outflow tract, *PV* pulmonary veins, *RV* right ventricle, *SAN* sinoatrial node, *SV* sinus venosus

field contribute to the arterial pole (outflow tract) and right ventricle of the heart tube (Fig. 1.2). The heart tube is associated with the embryonic dorsal mesocardium, which is thought to be disrupted during looping, only leaving contact at the arterial and venous poles. After looping, the heart tube consists of several segments: the left and right horn of the sinus venosus, the primitive atrium, the ventricular inlet segment, and the ventricular outlet segment [1].

Chamber differentiation occurs during further rightward looping of the heart tube, which results in positioning of the ventricles and the outflow tract of the heart in an anterior/ventral position, and of the atria in a dorsal/posterior position (Fig. 1.3). Transcriptional regulators (Nkx2-5, Tbx1, Tbx2, Tbx3, Tbx5, GATA4, Irx3 along with many others) and signaling pathways (including Notch, WNT, Bone Morphogenetic Protein [BMP], and Retinoic Acid) control chamber differentiation and formation of septal structures, the valves, and the great arteries [2, 3]. Electrical activity occurs early during development of the heart in conjunction with further differentiation of the simple heart tube into a four-chambered structure [4].

There has been much controversy with regard to the origin of the specialized myocardial tissue that leads to the development and expression of the conduction system. Current understanding suggests that cardiac myocytes, rather than neural crest cells, for example, are the progenitors of specialized conduction tissue. These findings were primarily supported by retroviral reporter gene transfection lineage studies [5–9]. The exact factors dictating this differentiation and development, however, remain to be elucidated, but it appears that neuregulin plays a crucial role in this differentiation process [10–16].

In a brief review in 1976, Wenink and colleagues proposed that there were four rings of specialized tissue in the embryo that could be

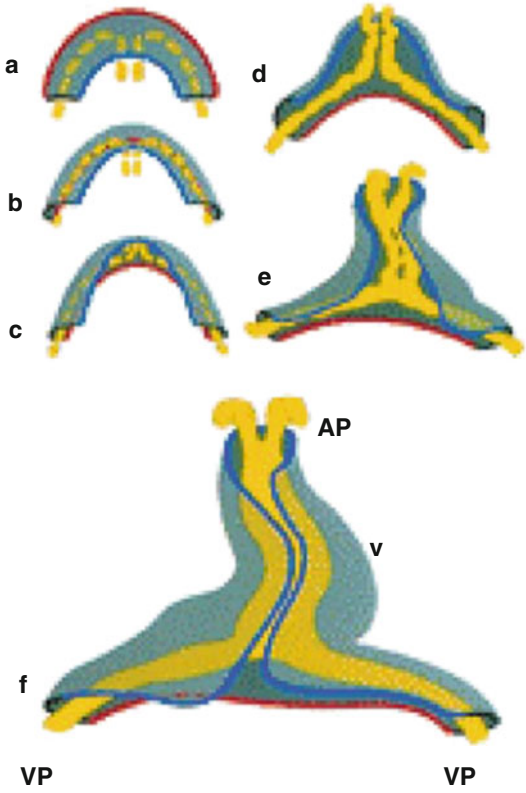

Fig. 1.2 Transformation of the flat cardiogenic crescent into a cardiac tube is displayed. During this process, the red outer contour of the myocardial crescent (*gray*) folds around the fusing endocardial vesicles (*yellow*) and passes the blue inner contour of the crescent, thereby forming the cardiac tube. *AP* anterior pole, *VP* venous pole, *V* future ventricle [Adapted from AFN Moorman et al., Development of the cardiac conduction system. Circulation Research 1998; 82:629–644. With permission from Wolters Kluwer Health]

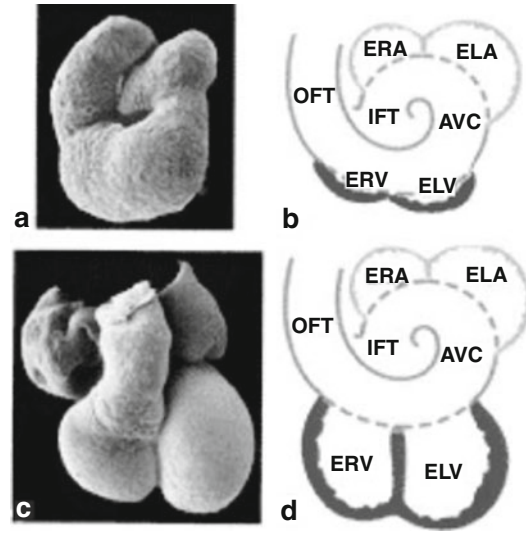

Fig. 1.3 Scanning electron photomicrographs (**a** and **c**) and schematic representations (**b** and **d**) of a 3-day embryonic chicken heart, where the first signs of the ventricles emerge (**a** and **b**), and of a 37-day embryonic human heart with clearly developed ventricles (**c** and **d**). *ERA* embryonic right atrium, *ELA* embryonic left atrium, *ELV* embryonic left ventricle, *ERV* embryonic right ventricle. The atrial segment is indicated in *blue*; the ventricular segment, in *red*; and the primary heart tube, encompassing the flanking segments, IFT, AVC, and OFT, as well as the atrial and ventricular parts, in purple [Adapted from AFN Moorman et al., Development of the cardiac conduction system. Circulation Research 1998; 82:629–644. With permission from Wolters Kluwer Health]

distinguished from the surrounding myocardium once looping of the heart had occurred [17]. These four rings (Fig. 1.4) were thought to mark transitional zones of the heart and included: the sinoatrial ring, between the sinus venosus segment and the primitive atrium; the atrioventricular ring, between the primitive atrium and primitive left ventricle; the primary ring or fold, that separates the primitive left ventricle from the primitive right ventricle; and the ventriculoarterial ring, at the junction of the primitive right ventricle and the truncus or putative outflow tract of the heart [1] (Fig. 1.3). It is thought that during completion of looping of the primitive heart tube, these four rings come together in the inner curvature of the heart and with further differentiation; part of this tissue loses its specialized character. What remain of the rings become the definitive elements of the mature conduction system. According to this theory, the sinoatrial ring contributes to the formation of the sinoatrial node; both the sinoatrial ring and the atrioventricular ring contribute to the atrioventricular node. The primary ring gives rise to the His bundle and bundle branches while the ventriculoarterial ring regresses almost entirely.

Studies in the 1990s used the expression pattern of a neurofilament-like protein as a marker for the developing conduction system. The presence of neurofilament-like protein was used to demonstrate a ring at the sinoatrial and atrioventricular junctions, and in ventricular components of the developing conduction system, which were

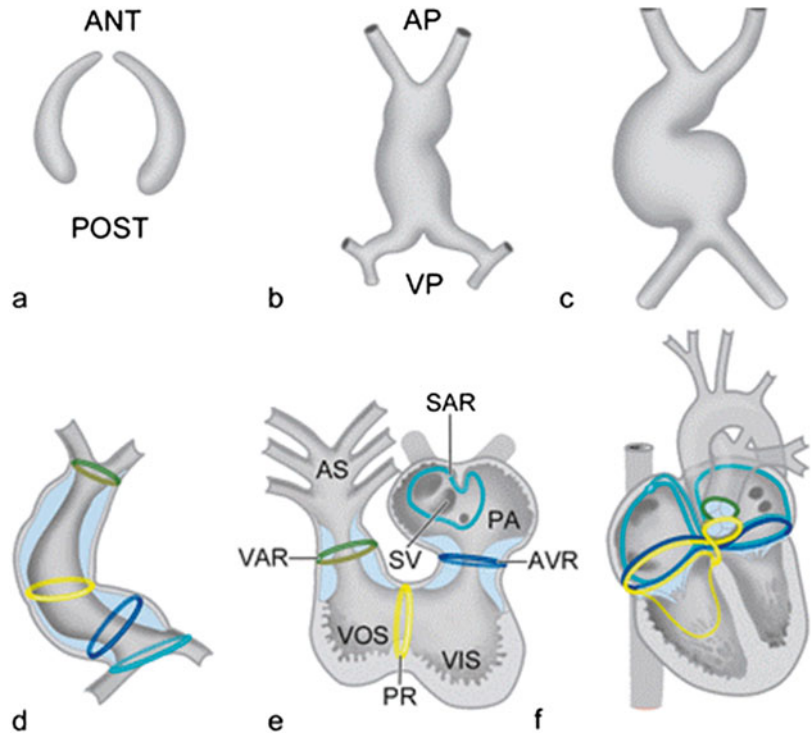

Fig. 1.4 Schematic representation of the bilateral formation of the cardiogenic plates, which are derived from the splanchnic mesoderm (**a**). The bilateral plates fuse and form an initially straight heart tube (**b**) that starts looping to the right (**c**, **d**). After looping, the so-called transitional zones or rings can be recognized in the heart that are positioned in between the putative cardiac chambers, i.e., the sinoatrial transition (SAR), the atrioventricular transition (AVR), the primary ring (PR), and the ventriculoarterial transition (VAR) (**e**). Position of these rings during further cardiac development (**f**). *Ant* anterior, *AP* arterial pole, *AS* aortic sac, *PA* primitive atrium, *post* posterior, *SV* sinus venosus, *VIS* ventricular inlet segment, *VOS* ventricular outlet segment, *VP* venous pole. **a–c** [Adapted from Gittenberger-de Groot AC, Bartelings MM, Deruiter MC, Poelmann RE. Basics of cardiac development for the understanding of congenital heart malformations. Pediatric Research 2005;57:169–176. With permission from Nature Publishing Group]

distributed in the ventricular subendocardium and connected to the atrioventricular ring [18–22]. In contrast to the theory that local cells undergo specialized differentiation, other studies suggest that conduction tissue cells (of the rabbit heart, for example) may originate from a population of neural crest-derived cells migrating from the branchial arches into the developing heart [20].

As these conflicting theories continued to be investigated, several immunohistochemical and molecular markers for cardiac conduction system development were used to support the hypothesis that conduction system cells differentiate from local cells. Even though none of the immunohistochemical markers are truly specific for labeling specialized conduction system cells, supportive evidence seemed to favor the "four ring theory." Using a monoclonal antibody to HNK1 antigen, for example, investigators demonstrated findings consistent with the notion that rings of conduction system tissue exist and undergo further differentiation (Fig. 1.5). HNK1 is predominantly expressed in the developing sinoatrial and atrioventricular junction of the conduction system, and the expression pattern seems to correspond with the rings described early on by Wenink. In human embryos, antibodies to HNK1 antigen stains the sinoatrial node, the internodal myocardium in the right atrium, the right atrioventricular ring with a future posterior and

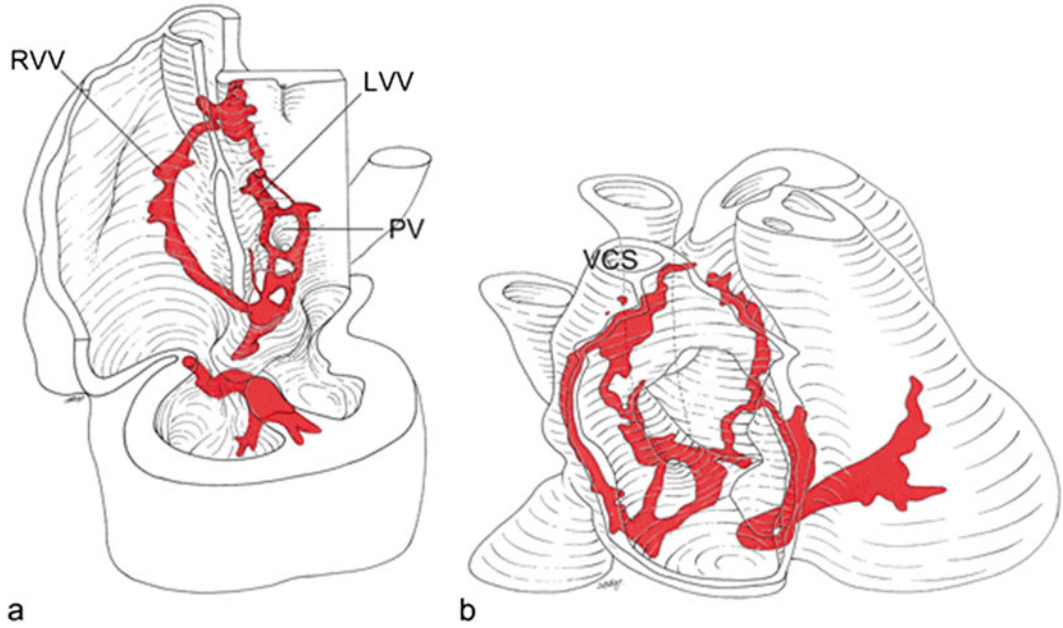

Fig. 1.5 HNK1 stains the sinoatrial node, the internodal myocardium in the right atrium, the right atrioventricular ring with the posterior and anterior atrioventricular nodes, a retroaortic ring, the His bundle, and the bundle branches in human embryos. Furthermore, the myocardium surrounding the primitive pulmonary vein demonstrates transient staining. *RVV* right venous valve, *LVV* left venous valve, *PV* pulmonary veins, *VCS* superior vena cava [Adapted from Blom NA, Gittenberger-de Groot AC, DeRuiter MC, Poelmann RE, Mentink MMT, Ottenkamp J. Development of the cardiac conduction tissue in human embryos using HNK-1 antigen expression—Possible relevance for understanding of abnormal atrial automaticity. Circulation 1999;99:800–806. With permission from Wolter Kluwers Health]

anterior atrioventricular node, a retroaortic ring, the His bundle, and the bundle branches. It appears that the myocardium surrounding the primitive pulmonary veins also demonstrates transient staining of HNK1 [23].

Podoplanin is a 43-kd, mucin-type transmembrane glycoprotein that is found outside the heart in several organs and tissues [24–32], such as osteoblasts, the nervous system, epithelia of lung, eye, esophagus, and intestine, mesothelium of the visceral peritoneum; the podocytes of the kidney; and lymphatic endothelium [33–36]. It is thought that podoplanin expression in the developing heart is a marker for the developing sinus venosus myocardium, supporting its development from the posterior heart field. Podoplanin is expressed in the areas that are in close contact with the sinoatrial nodal myocardium and in the underlying mesenchyme adjacent to the cardinal veins. It appears that podoplanin-positive mesenchyme differentiates into myocardium that stains negative for Nkx2.5. During cardiac development, podoplanin is expressed in myocardium along bilateral cardinal veins and in both the right-and left-sided sinoatrial nodes. This expression is maintained on the right as part of the right sinus node and right-sided venous valves, at the base of the atrial septum, the posterior atrioventricular canal, the atrioventricular nodal region, and the His-Purkinje system; it is opposed by the expression of Nkx2.5. Also, during later developmental stages, podoplanin is expressed in the pulmonary veins. In podoplanin negative mice, myocardial components around pulmonary veins are reduced and there is underdevelopment of the atrial septum [28, 37]. It appears that podoplanin plays a critical role in myocardial tissue associated with the sinus node and that abnormal epithelial-to-mesenchymal transformation of the coelomic epithelium due to up-regulated E-cadherin and down-regulated RhoA impose abnormalities in the formation of cells that form the sinus venosus [28].

Even though the development of anatomical structures supporting the specialized conduction system offers insight into genesis of the conduction system, functionally, the development of impulse generation and propagation remains to be fully understood. In the mature heart, the sinoatrial node is the primary pacemaker of the heart, and impulse propagation occurs through the atrioventricular node and specialized His-Purkinje system. Impulse propagation, itself, can be further divided into fast, as in the His-Purkinje system, and slow as seen in myocardial tissue, and slower yet, as seen in the atrioventricular node [6]. Different animals reveal complex variations in the organizational and functional components of the conduction system [7, 8, 38–40]. For example, the study of the chick embryo allowed understanding of a pacing generator around 25–35 h of development in the posterior most part of the tubular heart [41]. Similarly, pacing activity is noted around 7.5 days and 21 days in mice and humans, respectively [42]. At this time, the heart consists of a simple heart tube and the initial contractions are slow and rhythmic [43], but establish unidirectional flow and posterior to anterior polarity [44–48]. These peristaltic contractions can be recorded, inscribing a sinusoidal ECG [49].

Furthermore, there appear to be transient expression of key transgenic markers, timed chronologically, that determine developmental fate of myocardial cells. For example, the heart tubes in zebrafish, chicks, and mice appear to have retinoic acid-sensitive markers along the heart tube that dictate formation of atrial tissue [50]. Retinoic acid appears to control atrial-specific gene expression and exclusion of retinoic acid from ventricular tissue precursors seems essential for correct specification of the ventricular muscle development. In addition, transmembrane hyperpolarization-activated cyclic nucleotide-gated family of ion channel subunits plays a key role in impulse generation supporting pacemaker activity, both in the embryo and the adult human heart [49, 51–53]. Other genes also play a role in impulse generation as can be seen in studies that show that knock-out of the NaCa exchanger gene causes mortality due to inhibition of pacing function in the tubular heart [54]. Along with further differentiation, the developing atrial and ventricular myocardial cells acquire high conductance gap junctions that can then support rapid transmission of an electrical impulse by rapid proliferation and up-regulation of genes. These working myocardial cells have increased mitochondria and increased sarcomere components.

In contrast to rapidly proliferating myocardial tissue, cells in the atrioventricular canal area retain their slow proliferation rates, and also retain their “embryonic-like” mode of conduction, which is much slower [51, 55, 56]. In association with chamber formation, slow wave propagation producing peristaltic contractions are replaced by rapid depolarizations (and contractions) of cells of the atrium and ventricles, inscribing an ECG that resembles the one of the mature heart. These changes seem to occur in parallel with anatomical looping of the heat tube. Differential expression in conduction velocities of the conduction system components in the mature heart accompanies looping. These structural changes parallel a delay in conduction time in the mature atrioventricular node [57–59]. In fact, in the adult myocardium, the impulse proceeds from the sinus node to the crux of the heart at 0.1–1.0 m/s and is slowed at the atrioventricular node to 0.01–0.05 m/s, increasing its velocity to 2–4 m/s in the His-Purkinje system, with a decrease to 0.3–1.0 m/s in the ventricular myocardium [4]. Thus, sequential contraction of the atrial and ventricular chambers in higher species is dependent on the specific functional development of atrioventricular delay [5, 6, 16, 43, 60, 61]. This delay can be seen at 42 h of development in the chick and at 8 and 25 days in the mouse and human [6, 7], respectively. Furthermore, in the looping heart, there are two other areas of relatively slow conduction: the sinoatrial area and the outflow tract area. This slow conduction is associated with the expression of connexin 45, which is characterized by high voltage sensitivity and low permeability [62–65]. Knock-out mice of the connexin 45 gene result in death from heart block at looped, tubular stages of heart development [66, 67].

The His-Purkinje system is the last component of the conduction system to differentiate. In mammals, differentiation of the His-Purkinje system is quite advanced, resulting in markedly efficient and coordinated myocardial activation and associated myocardial contraction [5, 8, 68]. Retroviral lineage studies suggest that central and distally located components of the His-Purkinje system differentiate separately, but then link together during development [7]. In 1999 and 2000, differentiation of the His-Purkinje system in ventricular myocytes was found, in the chick embryo, to be induced by endothelin-1, secreted from adjacent coronary arterioles [69, 70]. This particular finding was not noted in the mouse, however. Similarly, some in vitro evidence suggested that neuregulin-1 played a role [15, 71, 72] in His-Purkinje development. In addition, cellular studies observed that there is a switch in activation sequence in the developing heart. The emergence of the mature His-Purkinje system in the developing chicken embryo had been studied using anti-polysialylated neural cell adhesion molecule (PSA-NCAM) and the HNK1 antibody against a sulfated carbohydrate epitope (antigen). The appearance of the mature form of the His-Purkinje system coincided with the onset of the mature electrophysiological patterns of ventricular activation. These data suggested that, at the completion of ventricular septation, the His-Purkinje system undergoes critical structural and functional transitions that impacted the global pattern of impulse conduction and contraction of the developing four-chambered heart [73, 74]. Using cardiac conduction system-lacZ line of reporter mice, several investigators tested the ability of endocardial-derived and secreted (paracrine) factors to convert contractile cardiomyocytes into conduction system cells. It appeared that neuregulin-1, a growth and differentiation factor essential for ventricular trabeculation was sufficient to induce ectopic expression of a lacZ conduction marker. In the mouse model, this inductive effect of neuregulin-1 was restricted to a window of sensitivity between 8.5 and 10.5 days fertilization. Thus, it appeared that endocardial-derived neuregulins may be responsible for inducing murine embryonic cardiomyocytes to differentiate into cells of the conduction system [15]. In a similar manner, Gassanov et al. described differentiation of atrial-derived cardiomyocytes to a pacemaker-like phenotype induced by endothelin-1, but not associated with neuregulin [72].

Cellular Development of "Nodal" Phenotype

In the mature heart, the atrioventricular nodal myocytes display a variety of embryonic characteristics. Despite these characteristics, nodal cells are poorly distinguishable from surrounding myocardium in the embryonic heart. During gestation and development, nodal cells retain organized actin and myosin filaments and a poorly developed sarcoplasmic reticulum. Nodal cells also continue to express different structural and cellular markers, which are species specific. Several classes of markers have been identified including connexins, specific contractile proteins, desmin, and neurofilaments. These specific markers provide an opportunity for the study of conduction system development. During development, unique characteristics of nodal cells include the expression of higher amounts of calcium-release channel/type-1 inositol triphosphate receptor, gamma enolase, alpha 1 and alpha 2 units of the sodium pump, G-protein alpha subunit, and angiotensin II receptor [26, 75–86]. The role of these differences is unclear at this time. Antibodies to carbohydrate markers such as the polysialylated neural cell adhesion molecule and HNK1 have been used to study the development of specific regions of the specialized conduction tissue. The role of some of these key factors is reviewed below.

The T-Box Family of Transcription Factors

The T-box transcription factors Tbx2 and Tbx3 are expressed in the cardiac inflow tract, the atrioventricular canal, the outflow tract, and inner curvature of the heart during development.

These factors are transcriptional repressors of chamber formation. Both Tbx2 and Tbx3 suppress the genes Nppa and connexin 40, present in working myocardium [55, 76, 87–89]. In general, expression of Tbx2 and Tbx3 is observed in slow-conducting areas, but also in the His bundle and the proximal part of the bundle branches. The expression of Tbx2 decreases from early fetal stages, whereas the expression of Tbx3 increases.

In the developing heart, expression of Tbx3 is observed in the sinoatrial node and atrioventricular node, but also in the internodal myocardium, in the atrioventricular cushions and in the His bundle and proximal bundle branches [88, 89]. Homozygous Tbx3-mutant mice display a syndrome known in humans as ulnarmammary syndrome and display early embryonic mortality, presumably due to severe compromise of the yolk sac [90]. The role of Tbx3 in controlling the sinoatrial node gene program has also been described [88, 89]. Tbx3 is expressed in the developing and mature sinoatrial node and is required to suppress the expression of genes regulating atrial differentiation. Furthermore, Tbx3 can induce ectopic pacemaker sites in the atria [88, 89].

The T-box transcription factor Tbx5 is expressed in the developing the atrioventricular node, His bundle, and bundle branches [85]. Mice lacking Tbx5 display a cardiac phenotype that resembles the Holt–Oram syndrome, including atrial septal defects and conduction system abnormalities [2]. Tbx5 targets atrial naturetic factor (ANF) and connexin 40 as part of the fast-conducting components of the conduction system. In mice, Tbx5 haplo-insufficiency causes a maturation failure of conduction system morphology and function [85]. Tbx5 is required for connexin 40-independent patterning of the cardiac conduction system and it is thought that the electrophysiologic defects in Holt–Oram syndrome reflect a developmental abnormality of the conduction system [82]. Tbx18 is expressed in the sinus horns and is likely essential for the formation of the sinus venosus. In mice that are deficient for Tbx18, formation of the sinus venosus is disturbed [87].

Homeodomain Transcription Factors

The homeodomain transcription factor Nkx2.5 is expressed early in development, in the cardiogenic mesoderm and is present throughout the developing heart [91]. As part of an ongoing chamber formation program, Tbx5 and Nkx2.5 stimulate a variety of cardiac genes. During development certain regions in the linear heart tube remain embryonic in nature and do not develop into working chamber myocardium due to the presence of Tbx2. Thus, it appears that Nkx2.5 and Tbx2 form a repressor complex that suppresses genes that promote a chamber differentiation program. Tbx2 is expressed in the primary myocardium of the inflow tract, atrioventricular canal, and outflow tract. It appears that Tbx2 competes with Tbx5, and when Tbx2 is expressed in conjunction with Nkx2.5, it seems to act as a repressor of further differentiation.

The expression of Nkx2.5 is elevated in the differentiating atrioventricular conduction system, compared to its expression in the adjacent working myocardium. This expression correlates with the recruitment of cells to the developing atrioventricular conduction system [92]. In Nkx2.5 haplo-insufficient mice, there is hypoplasia of the atrioventricular node and His bundle, and the number of peripheral Purkinje fibers is significantly reduced [93–95].

Cardiac phenotypes of mutations in Nkx2.5 in mouse models resemble those in humans and include conduction defects [96]. It is known that Nkx2.5 is not expressed in posterior heart field-derived myocardium, including the sinoatrial node and the sinus venosus [87]. Furthermore, Nkx2.5 interacts with the connexin 40 promoter region and mice lacking Nkx2.5 demonstrate a significant decrease in connexin 40 expressions [97]. Nkx2.5 can form a complex with the transcription factor Tbx2 that is able to suppress ANF promoter activity in the atrioventricular canal, which may be a mechanism that helps to regulate some of the sites of chamber formation in the developing heart [91]. Nkx2.5 can also bind to Tbx5. This complex is an essential component for the activation of the atrial naturetic factor gene.

The homeodomain transcription factor Msx2, a downstream target of Pax-3/splotch (which is a key player within early cardiac neural crest development), is expressed in the developing central conduction system, but not the peripheral Purkinje fibers, in the chick. However, no abnormalities in the cardiac conduction system have been observed in Msx2-mutant mice [95, 98].

The homeobox gene Hop is strongly expressed in the atrioventricular node, His bundle, and bundle branches of the adult cardiac conduction system and Hop-null mice demonstrate conduction defects below the atrioventricular node, related to decreased expression of connexin 40 [99].

The homeodomain transcription factor Shox2 is expressed in the embryo in the craniofacial region, limbs, brain, and heart [100, 101]. In the heart, Shox2 can be detected early in the posterior region of the primitive heart tube. During further development, Shox2 is expressed in the sinus venosus myocardium, which includes the sinoatrial nodal region and the venous valves; expression is also observed in the primitive left and right bundle branches. Shox2 knock-out mice die between 11.5 and 13.5 days postfertilization and show severe hypoplasia of the sinus venosus myocardium of the posterior heart field, including a decreased size of the sinoatrial node region and hypoplastic venous valves. When Shox2 is absent in knock-out mice, aberrant expression of connexin 40, connexin 43, and Nkx2.5 is observed within the sinoatrial node, indicating abnormal differentiation of the sinoatrial node as well as disturbed pacemaker function. This finding is also noted in the node in zebrafish embryos [100]. Given these findings, it appears that Shox2 is important in recruiting sinus venosus myocardium, including the sinoatrial nodal region.

The bicoid-related homeodomain transcription factor Pitx2c is involved in directing left/right identity in the heart at the venous pole [83] and is probably involved in suppression of left-sided sinus node formation, as Pitx2c-deficient fetuses form sinoatrial nodes in both the right and left atrium [102, 103].

Id Family of Transcriptional Repressors (Helix-Loop-Helix Containing Transcriptional Repressors)

Early in cardiac development, the temporal and spatial expression of Tbx5 supports specification of cells for the conduction system. Tbx5-dependent expression of connexin 40 and presumably other molecules are required for the critical electrophysiologic properties of these cells. It is thought that Tbx5 directs the expression of certain genes, such as those for connexin 40, in the mature conduction system, after the primitive atrioventricular node, left bundle branch and right bundle branch have assumed their adult structures. This observation may explain why some Holt–Oram patients, for example, or Tbx5del/+ mice show an evolution of conduction system disease with age [90, 91].

The gene Id2 has been identified by serial gene expression analysis (SAGE) as having ventricular conduction system expression and is a downstream target of Tbx5 and Nkx2.5. Id2 negative mice demonstrate ECG features of abnormal interventricular conduction, such as left bundle branch block in newborn and adult knock-out mice. Furthermore, intracardiac recordings are consistent with abnormal intraventricular conduction within the bundle branches [85]. In situ hybridization demonstrated that Id2, expressed in the conduction system in wild-type hearts, is not expressed in compound Tbx5/Nkx2.5 hearts, indicating that ventricular conduction system-specific expression of Id2 is dependent on Nkx2.5 and Tbx5. These findings support a link between a patterning abnormality of the developing conduction system and a functional abnormality of the mature conduction system [85].

Basic Helix-Loop-Helix (bHLH) Transcription Factors

Fate mapping analysis has revealed that Mesp1 is expressed in almost all of the precursors of the cardiovascular system, including the endothelium,

endocardium, myocardium, and epicardium. Mesp1-nonexpressing cells were found to be restricted to the outflow tract cushion and along the interventricular septum. When the interventricular cells were examined by using the pattern of beta-galactosidase activity, approximately 20 % of the ventricular conduction cells within the intraventricular septum correspond to Mesp1-nonexpressing cells. These data suggested that the ventricular conduction system is of heterocellular origin [104].

The GATA Family of Transcription Factors/Zinc Finger Subfamilies

The GATA family is a relatively small family of transcription factors. For three of the six known vertebrate GATA transcription factors, a role in cardiogenesis has been identified: these include GATA4, GATA5, and GATA6 [105]. Expression of GATA4 is present in both the adult and embryonic heart, and its disruption results in cardiac dysmorphogenesis with early embryonic mortality [87]. A significant interaction among the different transcription factors was shown in a study that demonstrated that, next to Tbx3 and Nkx2.5, the connexin 40 promoter is also modulated by the cardiac transcription factor GATA4 [97]. In addition, GATA4 is expressed in Purkinje fibers of the adult chick heart [106]. GATA5 mRNA is observed in the pre-cardiac mesoderm of the primitive streak embryo. In the embryonic heart, there is expression of the GATA5 gene in the atrial and ventricular chambers that, during further development, becomes restricted to the atrial endocardium [107]. Furthermore, GATA5 is expressed in the endocardial cushions and in the cardiac conduction system, in the sinoatrial node, atrioventricular node, bundle of His, and left and right bundle branches [84]. Interestingly, the GATA5 gene is expressed in a dynamic fashion over time in the septum transversum and in the epicardial organ of the mouse and avian heart, giving rise to the (GATA5-expressing) epicardium [84]. The cGATA6 gene enhancer specifically marks components of the developing cardiac conduction system and atrioventricular node [78, 108], but not the more distal components of the cardiac conduction system. Expression of cGATA6 remains visible in the mature cardiac conduction system.

MinK/lacZ Knock-In/Knock-Out

The minK gene (also known as IsK and KCNE1) encodes a 129-amino-acid protein that modifies transmembrane electrical currents in the heart resulting from expression of the genes HERG and KvLQT1 [109]. Mutations in both HERG and especially KvLQT1 that encode the structural subunits for the channels involved in the cardiac delayed rectifier currents IKr and IKs, respectively, are the most common causes of congenital long-QT syndrome (LQTS) [109]. Disruption of the minK gene and integration of the lacZ gene results in β-galactosidase expression under the control of endogenous minK regulatory elements, which has been used to study the expression pattern of minK in mice. Disruption of the minK gene causes inner ear defects and QT interval prolongation in bradycardic conditions, the combination of which is known in the human as the Jervell and Lange-Nielsen syndrome [110]. MinK–/– myocytes lack the delayed rectifier current IKs and demonstrate significantly reduced IKr, which indicates a role of minK in modulating both rectifier currents [109]. The spatial expression of minK-lacZ in the adult mouse heart has been shown to be coincident and closely related to the conduction tissue. The expression of minK-lacZ has been used to trace the embryonic development of the conduction system. Expression of minK-lacZ was first seen on the eighth embryonic day in the mouse. Subsequently, discrete rings were found at the sinoatrial, atrioventricular, interventricular, and ventricular–arterial junctions, and with time, the expression became restricted to boundary regions of the heart, such as the hinges of the leaflets of the pulmonary and aortic valves, the atrioventricular rings, and the venous valves, but was also noted in the definitive conduction tissue. In the postnatal mouse heart, areas retaining minK-lacZ positivity outside of the definitive conduction

tissues were thought to designate sites of origin of abnormal cardiac rhythms, suggesting that ectopic foci may derive from tissues that share a common developmental pathway with the definitive conduction system [111]. These observations suggest that the boundary regions between compartments, along with the atrioventricular conduction axis, share common developmental pathways and may support a certain arrhythmia expression later in life. Expression of minK-lacZ was not present at the site of the pulmonary veins [111].

Cardiac Conduction System lacZ Insertional Mutation

In 2000, it was noted that the random insertion of a lacZ gene into the murine genome unexpectedly resulted in a mouse line (named Cardiac Conduction System-LacZ [CCS-LacZ]) with expression of lacZ in the (developing) conduction system of the heart. Genetic mapping demonstrated that the transgene inserted into a regulatory region on mouse chromosome 7, altering transcription of several nearby genes including Slco3A1 [10–15, 47].

Regulatory elements from the gene Slco3A1 influenced cardiac conduction system-restricted reporter gene expression [112]. Members of the Slco family encode for organic anion-transporting polypeptides that mediate transport of natural substances (such as prostaglandins, bile salts, thyroid, and steroid hormones) as well as exogenous drugs (including digoxin, angiotensin-converting enzyme inhibitors, HMG-coenzyme A reductase inhibitors, methotrexate, and rifampin) across the cell membrane [113]. Considering the extent of the recombination observed in the CCS-LacZ model, it was thought that it would be likely that regulatory elements from more than one gene are involved [112]. In the CCS-LacZ mouse LacZ is expressed in all components of the developing cardiac conduction system, including the right and left venous valves and septum spurium of the sinus venosus and the putative sinoatrial node, the left and right atrioventricular ring, His bundle, bundle branches, and Purkinje fibers. CCS-LacZ is also expressed in the moderator band of the right ventricle, Bachmann's bundle, the retroaortic root bundle, and in the myocardial sleeve that develops around the pulmonary vein, areas related to arrhythmias in adults. Findings in several models support the hypothesis that the occurrence of cardiac arrhythmias in the heart, especially on the left atrial side, may be related to persistent or reactivated areas of developing cardiac conduction system [114–116].

CCS-LacZ expression was also noted in intraluminal endothelial cells, which are thought to be linked to the secretion of endothelial-derived factors involved in induction of cardiomyocytes to acquire a conduction system phenotype. Indeed, the endothelial paracrine factor neuregulin-1 has been demonstrated to induce ectopic expression of CCS-LacZ and, therefore, may play a critical role in recruitment of cells to the cardiac conduction system [15]. Timing of exposure to the endothelial factors may be crucial, as the inductive effect of neuregulin in the CCS-LacZ mouse was restricted to a window of sensitivity between E8.5 and E10.5 [15]. In the adult mouse heart, using serial sections of CCS-LacZ hearts, connexin 40 immunostaining (marking ventricular cardiac conduction system cells) could be co-localized with CCS-LacZ transgene expression in the atrioventricular node, His bundle, bundle branches, and subendocardial Purkinje fibers along the interventricular septum. In contrast to the developing heart and neonatal heart, cardiac CCS-LacZ expression was no longer present within the sinoatrial node in the adult mouse heart [117].

The Hyperpolarization-Activated Cyclic Nucleotide-Gated Cation (HCN) Channel Family

Four genes that encode HCN channels have been identified: HCN1, HCN2, HCN3, and HCN4. HCN channels carry an inward current, which is the depolarizing Na/K current if, that underlies cardiac pacemaker activity. In the adult heart, both HCN2 and HCN4 are expressed. During development, HCN4 is expressed as early as

E7.5 in the cardiac crescent [118, 119]. Interestingly, in the early heart tube, using optical mapping studies in the chick [120, 121], expression is observed bilaterally in the sinus venosus. Later in development, expression of HCN becomes asymmetrical and restricted to the right atrium, at the site of the developing sinoatrial node [118]. In the postnatal and adult heart, HCN4 is highly expressed in the sinoatrial node [118, 119]. HCN4 knock-out mice die between E9.5 and E11.5. These knock-out mice do not display mature pacemaker potentials, and thus, it is thought that HCN4 channels are required for proper pacemaker function of the sinoatrial node [119]. The expression pattern of HCN4 overlaps with the expression of markers of the posterior heart field, such as podoplanin and Shox2. The expression of HCN4 reflects the sinus venosus myocardium of the posterior heart field and becomes restricted to the sinoatrial node [118]. HCN2 is expressed in a broader distribution pattern than HCN4 and includes the ventricular myocardium, but is also moderately expressed in the sinoatrial node [118].

Connexins

The transmission of the electrical action potential is thought to occur primarily through gap junctions. Gap junctions are aggregates of membrane channels, composed of protein subunits named connexins that are encoded by a multi-gene family. Connexins hexamers make up connexons that then form the gap junction. Four different connexins are expressed in the mammalian heart including connexin 30.2, 40, 43, and 45. In the early myocardium, both number and size of gap junctions are small but they increase during development. The number of gap junctions remains scarce in the developing sinoatrial node and the atrioventricular node. The low abundance of connexin expression in the two nodes corresponds to their slow conduction velocities. This difference in connexin concentration has been an important marker for nodal-specific tissue. An abrupt rather than gradual increase in the number of gap junctions is found at the transition zone of nodal tissue to working myocardium. This boundary is thought to be due to a decrease in the number of nodal cells towards the atrial working myocardium rather than a gradient due to a change in molecular phenotype. Fast-conducting cardiac tissues in the atria express connexin 40 [122, 123] and slower conducting working myocardium express connexin 43 [124]. Connexin 45 seems to play a crucial role in delineating the conduction system during development and is seen in slow-conducting pathways, including the sinoatrial node and atrioventricular node during development [62–65, 124]. The expression of the different connexins varies among species but does provide an insight into the interplay and non-static nature of gap junction expression during development. For example, connexin 40 can be detected early in the mouse heart, where it is present first in the primitive atria and primitive left ventricle, and also in the primitive right ventricle, but not in the AV canal and interventricular septum. During development, together with the development of the specialized conduction system tissue, expression of connexin 40 becomes restricted to atrial myocytes and the ventricular conduction system [123]. Connexin 40 knock-out mice display an increased incidence of inducible atrial arrhythmias, and significant conduction delay in the infra-His and distal atrioventricular nodal conduction [62, 63, 122, 123, 125–129].

Connexin 43, in contrast, is first detected in the primitive ventricle and, some, in the atria and its expression increases and is present in the adult ventricular (working) myocytes [122, 123, 128]. Connexin 43 knock-out mice die at birth because of developmental defects in the pulmonary outflow tract, presumably resulting from defective migration of cardiac neural crest cells to this region [129]. In addition, cardiac-specific deletion of connexin 43 results in sudden cardiac death from spontaneous ventricular arrhythmias at 2 months postnatally, which suggests an important role for connexin 43 with regard to maintenance of electrical stability in the heart [130].

Connexin 45 is expressed in all compartments of the linear heart tube, including the inflow tract, atrioventricular canal, and outflow tract. Expression of connexin 45 decreases throughout

development and in the adult mouse heart, but remains present in the atrioventricular node, His bundle, and surrounding Purkinje fibers [62–65]. Connexin 45 knock-out mice demonstrate conduction block and die of heart failure [130].

Finally, connexin 30.2 slows impulse propagation through the atrioventricular node, which is important in preventing rapid conduction of an impulse into the ventricles [131–133]. In mice, in which the coding region of connexin 30.2 has been replaced by a lacZ reporter gene, a shortening of the QT interval by 25 % is seen [131].

Cytoskeletal Proteins

Nodal-specific developmental expression of contractile proteins such as myosin heavy chain and its isoforms, desmin and neurofilament, has been used to delineate the sinoatrial and atrioventricular nodes. However, inter-species variability in the staining of these markers does not produce sufficiently consistent data to draw definitive conclusions with relation to development or morphologic changes that are specific to the conduction system or its development and differentiation.

Atrioventricular Junction: Accessory Pathways/Mahaim Fibers

The atrial and ventricular myocardium including the atrioventricular canal is a continuous structure during embryogenesis of the heart tube and development of the four-chambered heart [134]. As demonstrated in the chick, accessory atrioventricular myocardial continuities may persist in the embryo until later stages, causing premature activation of the ventricles even after septation has occurred [135]. In normal adult cardiac conduction, the atrioventricular node-His bundle is the only functional atrioventricular conduction tract between the atria and ventricles. Rarely, accessory myocardial bundles or pathways connecting atrial and ventricular myocardial tissue persist, thus bypassing the insulating function of the atrioventricular groove [134] resulting in the well known in Wolff–Parkinson–White syndrome in humans [134]. A rare form of an accessory pathway is a right-sided accessory bundle with atrioventricular node-like conduction properties, at one time thought to be Mahaim fibers, but more correctly are atriofasicular fibers [116, 134, 135]. Data derived from the CCS-LacZ mouse demonstrate that the occurrence of these rare fibers may be related to the embryonic development of the right ventricular inflow tract. The development of the right atrial/right ventricular connection and concomitant outgrowth of the right ventricle results in a division of the primitive left and right ventricles. This division results in the development of the right ventricular moderator band that forms a right-sided atrioventricular continuity, similar to a Mahaim fiber. Electrophysiological experiments supported the presence of a slowly conducting right-sided atrioventricular pathway [116]. Other rare anomalous fibers that bypass the normal atrioventricular node-His-Purkinje axis are nodoventricular fibers atrioventricular node-ventricular connection) and fasciculo-ventricular (His bundle or right bundle connection) fibers that can cause pre-excitation and, rarely, reentry tachycardia. They are known as Mahaim fibers. These forms of pre-excitation can result in atrioventricular reentrant tachycardia (Chap. 4).

To date, there are two mouse models of Wolff–Parkinson–White syndrome. Mutations in the gene PRKAG2 (that encodes the γ-2 subunit of the AMP-activated protein kinase) seem to be associated with the expression of Wolff–Parkinson–White syndrome [136, 137] in humans. Mice that carry a mutation in the PRKAG2 gene display ventricular pre-excitation and a phenotype identical to humans with the familial form of ventricular pre-excitation [138]. Another form of pre-excitation has been demonstrated where the postnatal development of myocardial connections through the annulus fibrosus of the atrioventricular valves in mice overexpressing the PRKAG2 mutation occurs [139]. In this type of pre-excitation, there seems to be accumulation of excessive amounts of cardiac glycogen, and disruption of the annulus fibrosus by glycogen-filled cardiomyocytes [140, 141]. This form of pre-excitation is associated with myocardial hypertrophy.

A specific deletion of the gene ALK3 in the atrioventricular canal, coding for the type 1a receptor for bone morphogenetic proteins in the atrioventricular canal during development, causes ventricular pre-excitation, also, supporting the notion that this gene is important for normal atrioventricular junction development [80].

Epicardial inhibition studies demonstrate that reduced periostin expression at the atrioventricular junction, results in disturbed development of fibrous tissue at the atrioventricular junction, persistent atrioventricular myocardial connections with resulting ventricular pre-excitation, which may be another mechanism explaining Wolff–Parkinson–White syndrome [135].

In contrast to arrhythmias associated with accessory pathways, several other arrhythmias have been described that originate from the tricuspid and mitral valve junctions [142, 143] or around the atrioventricular annuli. Atrioventri cular cells surrounding both the tricuspid and mitral annuli have been shown to resemble nodal cells in their cellular electrophysiology [144], and thus, could support arrhythmias similar to those intrinsic to the atrioventricular node.

In summary, evidence suggests that the specialized conduction system develops from further differentiation of local myocytes. The molecular signals for this differentiation are multiple, variable, interactive, and dose- and timing dependent. The exact stimulants for differentiation, selective cellular potency, and variable cell protein and channel expression and their roles in differentiation deserve further study.

Part II—Anatomy of the Mature Cardiac Conduction System

The specialized conduction system of the mature human heart consists of a single sinoatrial node, atrial and intranodal pathways, the AV node, and the His-Purkinje system, the latter includes the right and left bundle branches of the His-Purkinje system, and the peripheral His-Purkinje system. This section focuses on the anatomy of the conduction system and its relationship with the luminal working myocardium as both are developed, in parallel and in conjunction with each other.

All cells in the heart are capable of conducting an electrical impulse, but a special subpopulation of myocytes differentiates to support both generation and propagation of the cardiac impulse. Even though microscopic inspection provides considerable insight into the structure of the conduction system [145], this method is incomplete and does not fully define and delineate specialized tissue behavior with regard to intramyocardial behavior and interaction [146, 147].

In the mature heart, the sinoatrial (SA) node is the dominant pacemaker of the heart and lies in the right atrium at the superior vena cava/right atrial junction, one millimeter below the epicardium of the sulcus terminalis [148–150]. It was first described in the early 1900s [151]. The sinoatrial node has the shape of an inverted comma, descriptively containing a head, body, and tail [145, 152–155]; rarely, the sinoatrial node has a horseshoe-shaped structure [156]. It tapers both medially and laterally and bends backward towards the left and then downward [157]. Several authors document a paranodal area, where cells are of the node intermingle with atrial cells [158–160]. The sinoatrial node is supplied by a relatively large artery, which courses through and gives off branches to the sinus node and adjacent atrial myocardium. It originates from the right coronary artery about 55 % of the time and from the left circumflex artery in about 45 % of cases [156].

With regard to the atrial body itself, it is controversial whether preferential intranodal pathways exist [161, 162] because conclusive anatomic data is missing.

Even though evidence for preferential intraatrial pathways is missing, there appears to be preferential conduction or impulse propagation that may be associated with the underlying anatomic differences in muscle density, muscle fiber orientation, and/or the thickness of the right atrial wall and its pectinate muscles. Some authors argue that “specialized pathways” consisting of aggregations and or concentrations of myocardial muscle fibers, bridge the SA and atrioventricular nodes or the right atrium to the left atrium.

These authors propose three internodal tracts: the anterior, middle, and posterior internodal fibers. The anterior intermodal fibers are thought to have two components: Bachman's Bundle, which bridges right and left atrium and "descending branches," which descend in the intra-atrial septum. The middle internodal tracts also known as Wenchebach's bundle are thought to arise from the posterior portion of the sinus node and then descend within the intra-atrial septum, anterior to the fossa ovale. And the posterior internodal tracts, also known as Thorel's pathway, are thought to exit the sinus node posteriorly and then descend within the crista terminalis, traversing through the Eustachian ridge, entering the AV node, posteriorly, in the mouth of the coronary sinus [161, 162]. An alternate hypothesis is that intra-arterial conduction depends on gap junction density at the cellular level.

Because it was difficult to differentiate electrical myocardium from working myocardium, anatomically, in 1910, the German Pathological Society defined myocytes that were responsible for conduction to be associated with: 1—histologically discrete from adjacent contracting myocardium, 2—traceable from pathologic section to section, and 3—insulated to some degree from adjacent tissue by a fibrous sheath. These criteria, established in 1910 have remained intact, although the specified distinctions can now be evaluated with more detailed histochemical staining techniques [146]. These staining methods, subsequently, led to the study of electromechanical coupling [146, 159].

The atrioventricular node is located in the posteroseptal area, primarily on the right atrial side, at the apex of the region known as the triangle of Koch. This triangle is defined by a fibrous structure known as the Tendon of Todaro, the edge of the septal leaflet of the tricuspid valve and the edge of the mouth of the coronary sinus, which marks the base of the triangle. A second isthmus is present between the mouth of the coronary sinus and the septal leaflet of the tricuspid valve that is thought to support slow pathway contributions to the atrioventricular node. In the adult, the triangle measures 14–20 mm in its longest apex-to-base dimension. In children, as expected, the dimensions vary based on height, weight, body surface area age, and heart weight [163]. The atrioventricular node abuts the mitral valve annulus and tricuspid valve annulus with its posterior margin abutting the coronary sinus. Unlike the bundle of His, the atrioventricular node cannot be seen visually, nor does it generate a distinct, recordable signal during clinical electrophysiologic testing. The knowledge of its location is inferred during electrophysiologic testing in association with mapping techniques. The anterior portion or distal ends of the atrioventricular node blend with the bundle of His, which penetrates the central fibrous body. The atrioventricular node is thought to be a flattened, oblong structure with multiple extensions, some extending to the left atrium. The atrioventricular node is also thought to have extensions with a compact portion of the node existing more closely associated with the perimembranous portion of the ventricular septum. The atrioventricular node is usually supplied by an atrioventricular nodal artery, which arises from the right coronary artery in 90 % of cases and from the left circumflex artery in 10 % of the cases.

The bundle of His consists as an extension of the atrioventricular node. These extensions occur distal to the compact atrioventricular node. The bundle of His is characterized by fibers, which are organized in parallel channels or strands. These fibers are surrounded by a fibrous sheath more proximally and are, therefore, well insulated. The bundle of His penetrates the fibrous body and proceeds anteriorly descending towards the atrioventricular septum where it divides into the right and left bundle branches [164–166]. The compact atrioventricular node is thought to be buried inside the central fibrous body insulated by fibrous tissue continuing with extensions to the bundle of His and the bundle branches.

The right bundle is a relatively well defined and easily dissectible structure situated beneath the epicardium on the right side of the intraventricular septum. The right bundle branch proceeds along the free edge of the moderator band to the base of the anterior papillary muscles in the

right ventricle and along the septal band to the apex of the right ventricle and to the "breakout point" on the anterior surface of the right ventricle [167].

The left bundle passes down the left side of the intraventricular septum and emerges below the posterior cusp of the aortic valve. In contrast to the right bundle, the left bundle breaks up almost immediately into a number of small fan-shaped branches, which proceed down the smooth aspect of the left side of the intraventricular septum. The bundle contains two major branches including an anterosuperior division and a posteroinferior division. The anterosuperior division is relatively long and thin whereas the posteroinferior division is relatively short and thick. The anterosuperior division is closer to the aortic valve whereas the posteroinferior division supplies the posterior and inferior aspect of the left ventricle [146].

Using histochemical techniques, researchers have been able to identify tissue that supports slow conduction in the posteroseptal area and also at the base of the aortic valve. Further staining studies confirmed a "figure of eight" configuration of these areas as the aorta moved closer to the left ventricle during development. Remnants of this slowly conducting tissue are thought to play a role in patients in congenitally corrected transposition of the great arteries or other abnormal muscular connections between the atrioventricular junction [43]. Transcription factor, Tbx3, is thought to play a crucial role during development, preventing such cells from conversion to working myocardial cells [160].

The nervous system serves a regulatory role by way of an integrated balance of sympathetic, parasympathetic, and sensory nervous system signaling supporting the heart in its development. The mature heart is extensively innervated. The sympathetic nervous system releases endorphins to increase heart rate, conduction velocity, myocardial contraction, and relaxation. Regional differences in sympathetic innervation of the heart have been noted during development and also during diseased states and vary with regard to patterning, signaling, and relevance. It appears that neural crest cells migrate during the middle of gestation and give rise to sympathetic neurons. Thus, sympathetic neurons extend from the stellate ganglion to the heart and into the myocardium. Specialized conduction tissue is more abundantly innervated compared to working myocardium [168–171]. Quantitative immunohistochemical and histochemical techniques confirm that regions of the conduction system possess a significantly higher relative density of total neural population immunoreactivity for the general neuronal marker proteins. Initial sympathetic dominance in the neural supply to the human cardiac conduction system in infancy and its gradual transition into a sympathetic and parasympathetic co-dominance in adulthood correlate well with the physiologic alterations known to occur in cardiac rate during postnatal development. The finding of reduction in density of innervation of the conduction tissue with ageing is also in agreement with clinical and electrophysiological findings such as age-associated reduction in cardiac response to parasympathetic stimulation [172].

Ganglia are located at the base of both atria and ventricles with a higher nerve density on the endocardium and greater nerve thickness on the epicardium. Parasympathetic supply to the myocardium arises from branches from the right and left vagus nerves. The right vagus nerve supplies primarily the sinoatrial node, given that the sinoatrial node originates from the right horn of the sinus venosus. The left vagus nerve supplies primarily the atrioventricular node due to its origin from the left horn of the sinus venosus. The parasympathetic nervous system mainly releases acetylcholine to decrease heart rate, cardiac output, atrial contractility, and conduction through the atrioventricular node. Finally, sensory signals from the myocardium are transmitted via thinly myelinated A-fibers and unmyelinated C-fibers to the upper thoracic dorsal horn via the dorsal root ganglia; the role of these fibers has not been fully elucidated.

The growth, migration, and behavior of cardiac neurons are orchestrated by a complex interaction and modulation between neural chemo-attractants and chemo-repellants. A set of neurotrophic factors act as chemo-attractants

both in the peripheral organs and in the central nervous system. There are two basic groups: (1) the neurotroponin family which include nerve growth factor, brain-derived neurotrophic factor, neurotroponin-3, and neurotroponin 4/5; (2) the glial cell line-derived neurotrophic factor family, which include glial cell line-derived neurotrophic factor, neurturin and atremin which bind to glial cell line-derived neurotrophic factor receptors. Glial cell line-derived neurotrophic factor and receptor signaling are required for normal parasympathetic innervation.

Because it is important to have a sound understanding of the atrioventricular junction for successful diagnosis and management of many cardiac arrhythmias, the Cardiac Nomenclature Study Group has divided the atrioventricular junction into anatomically distinct and separate regions for description of accessory pathway location and better health care professional communication. In addition, it is important to appreciate developmental changes as these have important implications for the study of the electrophysiologic structures in the pediatric age group and in patients with congenital heart disease and assist in an approach to ablation of the underlying abnormal substrate.

Part III—Anatomy of the Conduction System in Congenital Heart Disease

Development of the AV node and His-Purkinje system depends on appropriate atrial and ventricular orientation and proper alignment of the atrial and ventricular septum with appropriate closure of septal defects. A number of congenital cardiac malformations can impact this development and lead to anatomic substrates that give rise to cardiac arrhythmias.

Atrioventricular Septal Defects

Atrioventricular septal defects (also known as atrioventricular canal defects or endocardial cushion defects) involving abnormal development of the endocardial cushions may be associated abnormalities in atrioventricular conduction [173, 174]. Because the crux of the heart is abnormally formed in these defects, the atrioventricular node is inferiorly and posteriorly displaced [175], situated anterior to the mouth of the coronary sinus at a site just below where the base of the triangle of Koch would have occurred if the crux of the heart were properly formed. A common His bundle extends along the lower rim of the inlet portion of the ventricular septal defect resulting in a posterior course of the intraventricular conduction network. Therefore, due to the extent of the defect; the classic ECG pattern inscribes a superior leftward axis (vector). In addition, if the patient has a coexisting right posterior accessory pathway and supraventricular tachycardia, the accessory pathway might lie very close to the posteriorly placed atrioventricular node. Application of radiofrequency ablation could jeopardize the integrity of the atrioventricular node-His-Purkinje system and may result in complete heart block. Cryoablative therapy would be an advisable alternative in that situation.

Atrial Septal Defects

Even though the conduction system forms normally with regard to its anatomical location and its relative position to the crux of the heart, atrial septal defects can be associated with conduction system disease. Patients with mutations of Tbx5 or Nkx2-5 can present with both defects in atrial septation and progressive central cardiac conduction disorder which can result in complete atrioventricular block. That Tbx5 and Nkx2-5 mutations can also lead to complete heart block in the absence of structural heart disease suggest that this transcriptional pathway is directly involved in conduction system formation and maintenance [93–95].

For Tbx5, several lines of evidence support this assertion. First, despite an intact ventricular septum, all Tbx5del/+ mice had malformations in the ventricular conduction system, usually affecting both the right and left bundle branches.

Second, there was no relationship between the specific type of atrial septal defect in Tbx5del/+ mice and conduction system abnormalities. The presence of a secundum or primum atrial septal defect did not correlate with the severity of morphologic defects in the central conduction system, and no statistically significant differential effect was observed on the PQ interval, QRS interval, or likelihood of right-bundle-branch block. Thus, Tbx5 appears to have a direct role in conduction system development independent of its role in structural heart development. Furthermore, the finding that Tbx5 is expressed at high levels in conduction system cells suggests that its conduction system requirement may be cell-autonomous. Connexin 40, a transcriptional target of Tbx5 that encodes a gap junction protein required for normal electrophysiologic function of the heart, was considered a potential cause for the patterning defects evident in the central conduction system of Tbx5del/+ mice. Similar to Tbx5del/+ mice, Cx40–/– mice demonstrate prolonged PQ intervals, prolonged QRS intervals, and in some cases right-bundle-branch block [126, 127, 175]. The degree to which the decrement in Cx40 transcription in Tbx5del/+ mice accounts for the functional conduction system abnormalities in Tbx5del/+ mice remains unclear. Recent findings demonstrate the critical importance of even limited Cx40 expression in Tbx5del/+ mice: whereas Tbx5del/+ mice usually live to adulthood, Tbx5del/+ /Cx40–/– mice die in utero.

Cx40 deficiency does not, however, explain the morphologic abnormalities of the central conduction system found in Tbx5del/+ mice. Normal morphology of the atrioventricular node, atrioventricular bundle, and bundle branches was present in all adult Cx40–/– mice, indicating that this gap junction protein is not required for the morphologic maturation or patterning of the central conduction system. These findings implicate yet unidentified genes downstream of Tbx5 in the patterning of the conduction system [176].

Likewise, animal models have determined that Nkx2.5 has a direct effect on conduction system formation and maintenance. It has been noted to interact with Tbx5 to cooperatively regulate expression of a number of genes including Cx40 [97]. Haplo-insufficiency of either Tbx5 (Tbx5del/+) or Nkx2.5 (Nkx2.5del/+) results in slowing of conduction in the His-Purkinje system and which is further impaired in mice that are haplo-sufficient for both [177]. Further studies have demonstrated that the role of Nkx2.5 is not limited to early conduction system development. Perinatal loss of Nkx2.5 results in contractile and severe conduction system deficits shortly after the gene is experimentally deleted [178] even if that occurs after completion of the structural development of the heart. The requirement for Nkx2.5 in the maintenance of a healthy conduction system was determined using a mouse model in which Nkx2.5 was deleted 2 weeks after birth [179].

Ventricular Septal Defects, Including the Tetralogy of Fallot

In patients with ventricular septal defects, the AV node is usually in its anatomically correct position [168, 171]. The penetrating His bundle and the His-Purkinje system run posteriorly along the rim of the ventricular septal defect and then penetrate and depolarize the myocardium normally.

The exceptions include ventricular septal defects that are inlet in type and, therefore, support a more inferior and posterior propagation of initial ventricular activation, similar to those seen in atrioventricular septal defects. The course of the common bundle or its branches relatively to the ventricular septal defect may exhibit a longer common bundle. In patients with either inlet, perimembranous, or outlet ventricular septal defects, the His bundle and its branches will typically be found on the lower crest of the defect, and will tend to deviate slightly towards the left side of the defect. Therefore, in postoperative patients, a ventricular septal patch may overlie the region of interest where a His bundle could be recorded. In these patients, the amplitude and frequency of a His signal may be variable and perhaps diminished.

Atrioventricular Discordance

Other abnormalities of the conduction system are associated with AV discordance either in biventricular hearts or in hearts with single ventricle physiology [180–192].

In these patients, the atrioventricular node may be duplicated, situated outside the triangle of Koch or elongated in morphology. Remnants of slowly conducting tissue are thought to play a role in patients with congenitally corrected transposition of the great arteries (CCTGA) or other abnormal muscular connections between the atrioventricular junctions [43] where anatomical malformations support expression of unusual locations of the atrioventricular node.

Usually, in CCTGA, the conduction system extends medially and runs along the right-sided mitral valve and pulmonary valve. If there is a ventricular septal defect, conduction usually occurs along the upper border of the septal defect. As such patients with CCTGA or ventriculoarterial discordance may have more than one atrioventricular node that penetrated the atrioventricular groove that could support sinus rhythm and, in some patients, reentrant tachycardia [189–192]. It is well known that the atrioventricular conduction system can be tenuous in these patients and may lead to the development of spontaneous complete atrioventricular block [193–196]. Approximately 3–5 % of patients with L-transposition, especially those with associated single ventricle are born with complete heart block with an overall risk of development of spontaneous heart block, thereafter of approximately 2 % per year.

Another type of AV discordance occurs in atrial situs inversus with D-loop ventricles. Although there is evidence to suggest embryologic development of more than one atrioventricular node in this malformation, the posterior atrioventricular node tends to persist. These patients will, therefore, have a left-sided triangle of Koch, but will usually have an associated atrioventricular node displaced posteriorly and inferiorly. If there is the presence of a ventricular septal defect, the conduction system will run along the inferior border of the septal defect [197–201]. These findings suggest that the atrioventricular node is associated primarily with the morphologic right atrium.

Atrioventricular Discordance or Transposition of the Great Arteries

Abnormalities of outflow, and other conotruncal abnormalities and septal defects remote from the crux of the heart, usually do not affect the position and the location of the conduction system. In complete or dextro-transposition of the great arteries without a ventricular septal defect, the location of the conduction system is undisturbed. The atrioventricular anatomy is normal and there is normal atrioventricular concordance, therefore, allowing for normal atrioventricular conduction system development. However, these patients, if repaired by an atrial switch operation (Senning or Mustard operations) are subject to postoperative rhythm complications (Chap. 8).

Tricuspid Atresia

In tricuspid atresia, the atrioventricular node is typically associated with the atretic tricuspid valve in the right atrium. Studies confirm that the compact atrioventricular node in tricuspid atresia is situated in the right atrium inside the underdeveloped and diminutive triangle of Koch. The orifice of the coronary sinus can still be identified as the base of the triangle, but the tricuspid valve may be small and difficult to identify. A very short common bundle is described running towards the central fiber body, which then descends along the septum. Should a ventricular septal defect be present, the conduction system tends to travel along the lower margin of the ventricular septal defect on the side of the septum between the rudimentary right ventricle and left ventricle. Because of the diminutive right ventricle, left ventricular activation is predominant, resulting in a left superior frontal plane QRS axis (vector).

Ebstein's Anomaly

Ebstein's Anomaly is associated with a normal atrioventricular node and triangle of Koch. However, because of anatomic distortions associated with displacement of the septal and posterior leaflets of the tricuspid valve in association with right atrial and right ventricular enlargement, identification of the normal anatomy may be difficult. In these cases, the coronary sinus may serve as an especially useful marker for the delineation of the triangle of Koch. Because the anatomic and electrophysiologic atrioventricular groove are not anatomically the same in patients with Epstein's anomaly, it is often helpful to perform a right coronary artery angiogram to define the anatomic atrioventricular groove that can then be used to, more accurately, deduce abnormalities of electrical conduction in association with underlying anatomic structures. Simultaneous intracardiac electrophysiologic and pressure recording can demonstrate electrophysiologic-hemodynamic dissociation (see Chap. 23, Fig. 23.14). This cardiac abnormality is often associated with one or more accessory pathways and carries with it a higher incidence of atrial arrhythmias as well. An understanding of the anatomy and an effort to delineate present distortions can be critical for successful ablation at the time of the electrophysiologic study.

Heterotaxy Syndromes

These syndromes encompass a complex set of defects associated with "sidedness" confusion of organs in the thorax and/or abdomen. Two general subgroups exist: those with right atrial isomerism or "bilateral right sidedness" (also known as the Asplenia syndrome) and those with left atrial isomerism or "bilateral left sidedness" (also known as the Polysplenia syndrome). Typical cardiac features of bilateral right sidedness include an intact inferior vena cava, unroofed coronary sinus, total anomalous pulmonary venous return, complete atrioventricular septal defect, ventricular inversion and/or transposition of the great arteries, or double outlet right ventricle with pulmonary stenosis/atresia. Features of bilateral left sidedness include interrupted inferior vena cava, total or partial anomalous pulmonary venous return common atrium, complete or partial atrioventricular septal defects, normally related great vessels and/or double outlet right ventricle with or without pulmonary stenosis. The mode of inheritance of heterotaxy syndromes remains uncertain. There is some suggestion that there may be autosomal dominant and recessive forms; the majority of cases appear to be due to mutations in genes that encode sidedness in association with environmental insults. In a large study of the electrocardiograms of 126 patients with atrial isomerism, 67 with left atrial isomerism and 59 with right atrial isomerism, the cardiac rhythm in patients with left atrial isomerism, with supposed "absence" of normal sinus nodal tissue, tends to exhibit a wide range of P-wave axes suggesting a variety of atrial pacemaker locations. In addition, patients with left-sidedness exhibit sinus node dysfunction (80 % at 10-year follow-up). Furthermore, there are some instances of atrioventricular nodal abnormalities (15 %), while there were no atrioventricular node abnormalities in patients with bilateral right-sidedness. In contrast, patients with right atrial isomerism, with supposed "bilateral" sinus nodes, tended to exhibit P-wave axes predictive of either a high right-sided (between 0° and 89°) or high left-sided (between 90° and 179°) atrial pacemaker location. In patients with Asplenia syndrome (bilateral right-sidedness), ventricular inversion is more common and, thus, these patients are subject to expression of complete heart block. The reported cases of atrioventricular block were spontaneous, but heart surgery places the conduction system at additional risk.

These anatomic variants can be associated with two compact atrioventricular nodes where both anterior and posterior nodal structures are present. Conduction can occur through both atrioventricular nodes with interaction between the nodes to support reentrant tachycardia (Mönckeberg sling). In these cases, the posterior node seems to be a more developed structure and ultimately forms the connection to the His bundle

[197, 198]. Catheter ablation can successfully treat these patients [190–192, 198–201]. This entity seems to be more common in patients with right atrial isomerism.

Conclusion

Multiple congenital and acquired abnormalities of the conduction system can occur, driven primarily by a broad array of multiple genetic signals. The complex interaction of many of these factors and elements, driven by genetics and its interplay with the environment, remains incomplete, and offers stimulating areas for further investigation.

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