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T for Two: T-Box Factors and the Functional Dichotomy of the Conduction System

David S. Park, **Glenn I. Fishman***

Leon H. Charney Division of Cardiology, NYU Grossman School of Medicine, New York, NY

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The cardiac conduction system (CCS) generates each heartbeat and orchestrates impulse propagation throughout the heart. The CCS consists of the impulse generating but slowly conducting sinoatrial (SAN) and atrioventricular (AVN) nodes and the rapidly conducting His-Purkinje system (HPS). Slow conduction allows for adequate preloading of the cardiac chambers, while rapid conduction ensures synchronous contraction of the cardiac chambers, maximizing cardiac output and reducing arrhythmia susceptibility.

Dictating the electrophysiology of these distinct CCS regions are transcriptional programs that drive the expression of ion channels, exchangers, regulatory subunits, and gap junctional proteins that impart either a slow conduction/high automaticity phenotype versus a rapid conduction/low automaticity phenotype. The T-box (*TBX*) transcription factors play a key role in tilting the scales towards a slow or fast CCS lineage. T-box factors can function as transcriptional activators or repressors and are known to be critical regulators of cardiac specification and differentiation. Seven *TBX* family members are expressed in the developing heart, four of which (*TBX1, TBX3, TBX5, TBX20*) have been linked to human congenital heart disease[1–3]. Tbx3 is a transcriptional repressor essential for driving nodal gene programming, whereas Tbx5 is a transcriptional activator important for fast conduction gene expression.

Tbx3 is expressed throughout the developing and mature CCS where it represses fast conduction genes and enhances automaticity by driving expression of the pacemaker channel Hcn4 and T-type calcium channels, including *Cacna1g*. In addition, Tbx3 maintains slow conduction by repressing expression of the fast cardiac sodium channel pore-forming subunit, $Na_V1.5$ (encoded by *Scn5a*), and the high conductance gap junction proteins Cx40 and Cx43 while also activating expression of the low conductance gap junction proteins Cx30.2 and Cx45. *Tbx3* displays critical dose dependency for proper differentiation and homeostatic maintenance of the CCS[4]. Graded loss of *Tbx3* manifests as worsening sinus node dysfunction and inappropriate expression of rapid conduction genes (*Scn5a*, Cx43,

*Correspondence to GIF at: 435 East 30th Street, Science Building 717, New York, NY | 10016, Tel: 212-263-3967 | Fax: 212-263-3972, glenn.fishman@nyulangone.org.

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Cx40) within the SAN region[4, 5]. Conversely, ectopic expression of Tbx3 in atrial myocardium suppresses rapid conduction gene expression and up-regulates SAN-enriched gene programming (*Hcn4*, *Gjd3*/Cx30.2, *Cacna1g*)[5]. Tbx5 acts cooperatively with the homeobox-containing transcription factor Nkx2–5 to drive a rapid conduction gene program (*Scn5a* and Cx40) in the HPS[6–10]. Formation of the proximal HPS, namely the His bundle and bundle branches, is critically dependent on the co-expression of Tbx5 and Nkx2–5, and combined haploinsufficiency of these two factors results in a specification defect of the proximal HPS[11].

The HPS represents a unique region of the CCS where Tbx3 and Tbx5 are both expressed, creating a tension between automaticity and rapid conduction. From a biophysical perspective, rapid conduction and automaticity are poorly compatible. The cardiac sodium current (I_{Na}), which is the principal determinant of rapid conduction in the HPS, requires the inward rectifier potassium current (I_{K1}) to maintain a hyperpolarized resting membrane potential to allow $Na_V1.5$ to recover from inactivation. Automaticity, which is maintained by Hcn4, is defined by diastolic depolarization of phase 4 (I_{funny} or I_f, pacemaker maker current) and does not allow a stable hyperpolarized membrane potential. Therefore, in order to prevent automaticity from keeping $Na_V1.5$ in an inactivated state, automaticity must be subdued, I_{K1} (Kir2.1 and Kir2.2, encoded by *Kcnj2 and Kcnj12*, respectively) must be robust, and $Na_V1.5$ expression must be abundant. This electrophysiology defines the HPS.

Moskowitz and colleagues[10] previously showed that HPS-selective *Tbx5* knockout (KO) ($Tbx5^{MinKCre\text{-}ERT2}$) mice manifest conduction disease and ventricular arrhythmias. Both *Scn5a* and Cx40 were significantly reduced in the HPS. Two Tbx5 responsive enhancers were identified, one in the *Scn10a* locus and another 15kb downstream of *Scn5a* that together drive *Scn5a* expression within the HPS[12]. In this issue of Circulation Research, Burnicka-Turek et al.[13] now explore how gene dosage of Tbx3 and Tbx5 jockey for automaticity vs rapid conduction, respectively, in the HPS. Consistent with the known paradigm for T-box factors, Tbx3 is more enriched in AVN cells while Tbx5 is more enriched in Purkinje myocytes. Using loss-of-function or gain-of-function models under the control of the $MinK^{Cre\text{-}ERT2}$ system, the authors show that Tbx5 haploinsufficiency or Tbx3 overexpression is sufficient to produce conduction disease in the HPS. Combined haploinsufficiency of Tbx5 and Tbx3 is able to rescue the conduction defect, indicating that gene dosage of these Tbox factors dictates the dominant electrophysiological phenotype in the HPS. HPS-selective *Tbx5* KO leads to a reduction in rapid conduction genes (*Scn5a*, Cx40, *Kcnj2*, *Kcnj12*, and *Kcnk3*) without a change in nodal gene expression. *Tbx5* KO Purkinje cells acquire a nodal type action potential (AP), which recapitulates other studies[14] that have shown that downregulation of I_{K1} is sufficient to uncover spontaneous diastolic depolarization of phase 4. Furthermore, the reduction in *Scn5a* coupled with a depolarized RMP decreases $Na_V1.5$ availability, negatively impacting phase 0 of the action potential and conduction in the HPS.

As shown previously[10], reduced Tbx5 expression increases vulnerability to ventricular arrhythmias. HPS-selective *Tbx5* KO hearts exhibit ventricular ectopy and ventricular tachycardia (VT), some with identical ventricular activation pattern as during sinus rhythm. Based on this observation, the authors speculate that at least some of the arrhythmic events

in mutant hearts may have originated from the His-Purkinje system due to enhanced automaticity. However, their surface and intra-cardiac recordings also show evidence of ectopy arising from outside the HPS, suggesting additional studies will be required to define the precise origin and mechanism of the ventricular arrhythmias in mutant hearts. It will also be of interest to study whether transitional cells at the Purkinje-ventricular myocyte junction contribute to arrhythmogenesis. In addition to the HPS, MinK is also robustly expressed in the right ventricular outflow tract[15], and so it is feasible that some of the ventricular arrhythmias seen in mutant hearts may have originated from this highly vulnerable region.

Lastly, Tbx5 targeted cis-regulatory elements were identified using Chip-seq in embryonic hearts at E14.5. Two Tbx5 binding sites were identified that were previously shown[12] to be *Scn5a* enhancers that are necessary and sufficient for *Scn5a* expression. Additional Tbx5 responsive enhancers were identified in *Ryr2* and *Kcnk3*, and both channels showed reduced expression in the ventricular conduction system of HPS-selective Tbx5 KO hearts. Although Ryr2, which mediates calcium release from the sarcoplasmic reticulum, has not been implicated in cardiac conduction, altered calcium handling could potentially play a role in the arrhythmic phenotype of mutant hearts. The two-pore domain potassium channel *Kcnk3* (Task-1), which has been implicated in QT prolongation and conduction disease in the HPS[16], likely contributes to the conduction and possibly to the arrhythmic phenotype albeit the extent to which is speculation. Taken together, the work of Moskowitz and colleagues confirms that all components of the CCS exhibit automaticity driven by Tbx3, but in the HPS, Tbx5 is necessary to superimpose a rapid conduction gene program onto Purkinje cells.

The importance of reinforcing a rapid conduction gene program in the HPS is evident by the number of pathways dedicated to *Scn5a* and Cx40 expression in Purkinje cells. Additional HPS-enriched transcription factors, including Etv1[17] and Irx3[18], promote *Scn5a* and Cx40 gene expression, and loss of either factor negatively impacts rapid conduction gene programming. Understanding how these factors ultimately work together to either promote Tbx5 expression or function will be important to fully delineate the gene regulatory landscape of rapid conduction. Only then can we define how disease processes dismantle these transcriptional networks to give rise to conduction disease and arrhythmogenesis.

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