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Setting the pace: Tbx3 and Tbx18 in cardiac conduction system development

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A vexing problem for clinicians in the management of children and adults with congenital heart disease is the management of the accompanying arrhythmias. In the recent decade, the mutational spectrum associated with human congenital heart disease has begun to come into focus.¹ The number of distinct genes implicated in human congenital heart disease is considerable, and a large portion of mutated genes encode transcription factors. Autosomal dominant mutations in NKX2.5 associate with primarily atrial septal defects, but importantly these defects are distinguished by the presence of atrioventricular nodal defects including heart block. The Holt Oram Syndrome, or heart-hand syndrome, arises from dominant mutations in Tbx5, a member of the brachyury family of transcription factors and is also associated with the development of atrioventricular nodal disease. Further investigation of these genes using mouse genetic models has begun to refine the detailed program required for development of specific components of the cardiac conduction system.

The mammalian cardiac conduction system is composed of the sinoatrial (SA) node, the atrioventricular node, the His bundle, the left and right bundle branches, and the ventricular Purkinje fibers. For many of these components, transcriptional regulators have been identified that are critical for their proper development or maintenance (see Figure 1). Nkx2.5 is required for the development and maintenance of the atrioventricular node.² In addition, the development of the atrioventricular node is also dependent on Tbx5 and Id2, while the distal ventricular conduction system requires Id2 and HOP.^{3,4} The precise delineation and maintenance of the conduction system likely requires more than just these factors.

In mammals, the SA node serves as the dominant pacemaker from its position in the right atrium near the junction with the superior vena cava. The SA node is nonuniform in cell content and function. Early morphological studies described three cell types: nodal, intermediate and elongated cells where nodal cells were thought to drive the pacemaker. Nodal cells were defined by their small shape and ovoid morphology. The mammalian SA node is also unique in having a head region and a tail or cauda yielding a comet shaped structure. The cauda was observed to have nearly all nodal cells with fewer intermediate and elongated cells.⁵ Previous work by Blaschke et al. suggested the importance of the homeodomain transcription factor Shox2 in the development of the SA node.⁶ The recent work of Christoffels and colleagues has advanced our understanding of the SA node by first clarifying the essential role of the transcription factor Tbx3 in specifying atrial versus SA node cells.⁷ Tbx3 is thought to function as a transcriptional repressor to suppress atrial myocyte phenotype.⁸ Mice lacking Tbx3 die during embryogenesis between e11.5 and e14. Tbx3 null mice develop multiple abnormalities including limb malformation and failure to develop mammary glands. Therefore, Tbx activity is not unique to the developing heart and conduction system. However, within the heart Tbx3 expression is required for normal size and function of the SA node. Critically, ectopic Tbx3 expression within

the atria is sufficient to induce ectopic pacemaker sites associated with suppression of normal atrial gene expression and induction of *Hcn4*. The mechanism by which suppression of atrial myocyte gene expression couples to promote the unique gene expression profile within the SA node is not clear. In this issue, the complexity of SA node development was defined further by demonstrating that *Tbx18* also contributes significantly by driving specification of the head and tail components of the SA node.⁹

The largest portion of the SA node is the head region, and in this issue Wiese et al. demonstrate that the loss of *Tbx18* is required for normal formation of the SA node head region.⁹ The authors first demonstrated that the large SA node head differs in its gene expression profile compared to the SA node tail. Specifically, the head expressed *Tbx18*, *Hcn4* and lacks *NKX2.5* expression. In contrast, the tail lacked *Tbx18* expression and demonstrated higher levels of *Hcn4* and low levels of *NKX2.5* expression. Normally in the mouse, the SA node head quadruples in size between e12.5 and e14.5. In *Tbx18* deficient embryos, the tail portion of the SA node developed at e12.5 but the head region was absent at this point. Two days later, the SA node head region remained largely unformed. This absence was not associated with a reduction in cell proliferation or an increase in apoptosis. In addition, the expression of *Shox2* was unaffected in *Tbx18* deficient embryos, suggesting that *Shox2* is upstream of *Tbx18* or in a parallel pathway in the transcriptional regulation of SA node specification. It will be of interest to determine the pattern of expression of *Tbx3* and *Tbx18* in *Shox2* deficient embryos.

The origin of the cardiac conduction system, especially the SA node, has been uncertain. Conduction system cells may develop directly from mesodermal precursors that migrate into the heart and then differentiate into conduction system. Fate mapping experiments suggest that the cells that contribute to the SA node do not develop from atria in that they do not transition through a phase with a clear atrial gene expression program.⁷ Consistent with these previous observations, Wiese et al. generated animals expressing cre recombinase in the *Tbx18* locus combined with the *Rosa26* indicator allele to mark cells that during their developmental history transiently expressed *Tbx18*. In these experiments, it was shown that SA nodal head cells uniquely expressed *Tbx18* while the neighboring atrial myocytes showed no evidence of having ever had *Tbx18* expression. These data are consistent with a program where mesenchymal cells migrate into the atria and develop into SA nodal cells without first expressing atrial genes or adopting an atrial myocyte fate. Interestingly, in these fate mapping experiments, SA node cauda was found to show evidence of having transiently expressed *Tbx18*. Therefore, both *Tbx18* and *Tbx3* are required for differentiation and continued specification of the normal SA node.

The role of *Tbx3* in the conduction system extends beyond the SA node since *Tbx3* has also been shown to be required for the normal formation of the atrioventricular node. Loss of *Tbx3* leads to ectopic expression of connexin 43, atrial natriuretic protein, *Tbx18*, and *Tbx20* within the bundles. Misexpression of these genes supports the idea that *Tbx3* functions as a transcriptional repressor where in its absence atrial genes are expressed.^{10,11} Since fate mapping experiments for the SA node suggest that mesenchymal cells directly develop into nodal cells, these data together support a role where progenitor cells have multipotency and the ability to differentiate into conduction system as well as atrial and ventricular myocytes. In this model, distinct subsets of transcription factors would promote which fate the progenitor cell adopts. At which point or whether this multipotency is lost remains to be seen. These pathways are likely needed to successfully direct stem cells for heart repair, whether mediated by embryonic stem cells or adult progenitor cells. *Tbx18*-positive cells originating from the proepicardial organ migrate and contribute to the epicardium, and then following that differentiate into many different cell types within the heart that include not only atrial and ventricular myocytes but also fibroblasts and smooth muscle cells.¹² Thus, *Tbx18* acting in

concert with other factors, including Tbx3, form regulatory units that specify the cell types within the heart.

The ablation of Tbox genes in these mouse models renders loss of function but does allow for genetic compensation by other gene products. Human mutations differ from those in mice since they typically are point mutations associated with either partial loss or gain of function and are compatible with survival. In the human cases, more subtle changes in levels of protein expression or in protein function, such as interaction with binding partners, may affect heart development or produce disease of the mature heart. Pacemaker function within the heart varies with age; newborn humans have very high heart rates. Heart rate, including both resting and exercise induced, declines with age in a predictable fashion. The incidence of sinus node dysfunction and atrial fibrillation dramatically increases with age. The lifetime risk of developing atrial fibrillation is as high as 1 in 4, and sinus node dysfunction is responsible for nearly half of all pacemaker insertions.^{13,14} The Tbox genes may point to genetic markers useful for predicting risk of cardiac conduction system disease and guiding placement of devices to treat these disorders. Of note, Tbx18 harbors three nonsynonymous single nucleotide polymorphisms including one with high frequency in the population.¹⁵

Transcription factors, including Tbox factors, drive expression or repression of a constellation of critical regulatory genes, most of which have not yet been identified. These target genes are also essential in specifying and maintaining the conduction system. The genes that are regulated by Tbx3 and Tbx18 are largely unknown, but are clearly essential in determining the sharp delineation between a functioning SA node and the neighboring atrial myocytes. The Tbox factors are front and center in setting the pace of conduction system function.

Abbreviation

SA, sinoatrial.

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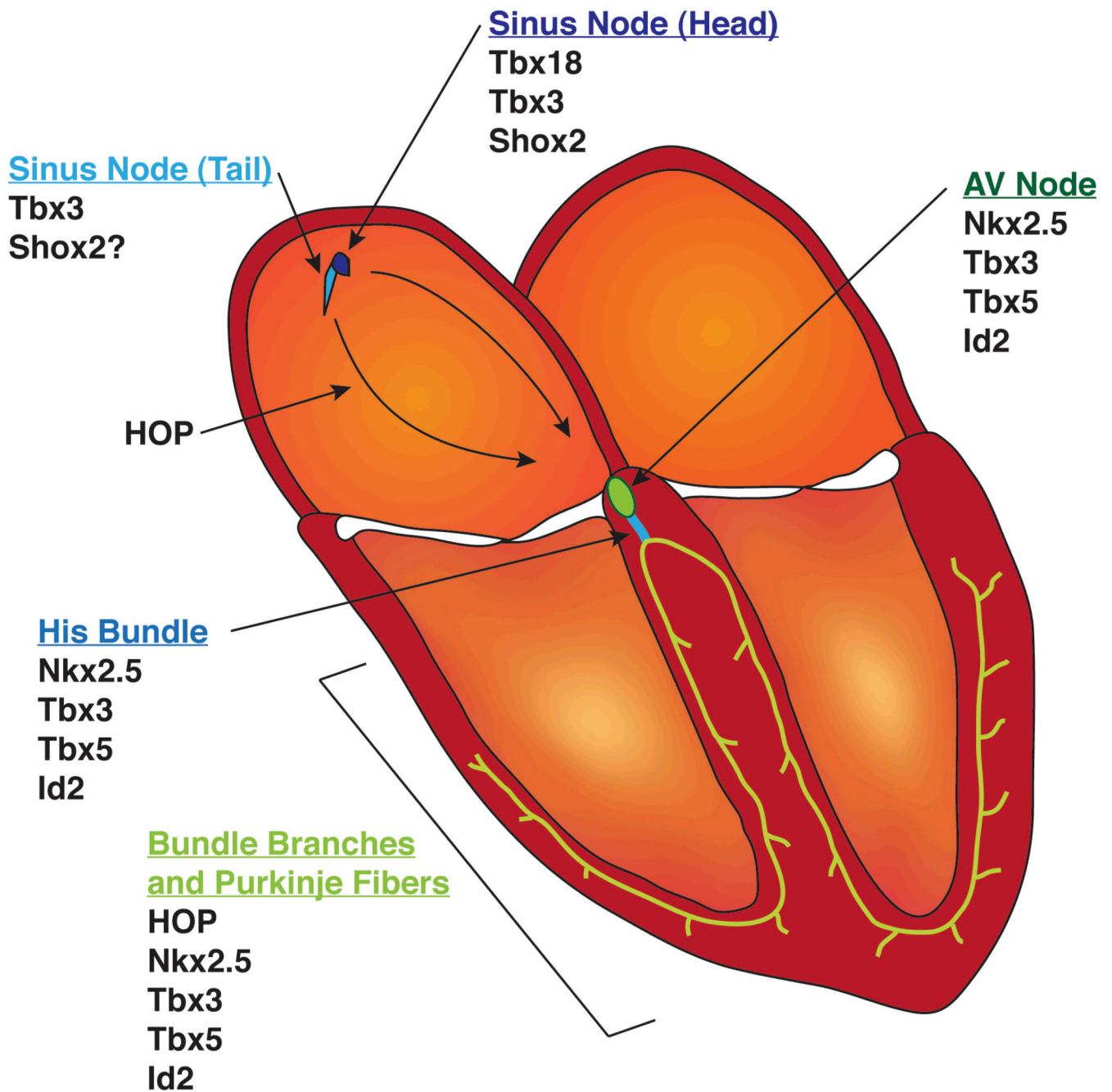


Figure 1.

Transcription factors required for cardiac conduction system development. Shown above is a schematic of the conduction system of the mammalian heart including the sinus node (blue), atrioventricular node (green), bundle of His (light blue), bundle branches and Purkinje fibers (yellow). The known transcription factors regulating the development of these components are indicated.